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THE MODE OF ACTION OF VERITOL. By AUGUST REIS. From the Department of Pharmacology, University of Edinburgh.

(Received for publication 5th June 1939.)

VERITOL, or β-(p-oxyphenyl) isopropylmethylamine, a synthetic drug, is a member, pharmacologically and chemically, of the adrenaline group. Its action on the circulatory system has recently been widely investigated, and the different workers agree in stating that it causes a prolonged rise in blood-pressure, but do not agree as to the quantitative relations between dosage/duration and dosage/extent of rise of blood-pressure. Moreover, explanations put forward as to the cause of rise of blood-pressure differ widely.

Rein [1937] and Heymans and Bayless [1937] state that the rise of B.P. caused by veritol is due to a greater filling of the vessels by an increase of the minute volume and not to an increase of the peripheral resistance to the flow. Lindner [1937] has come to a similar conclusion. Another group of authors, including Zipf [1937], Turchetti [1938], and Scaffidi [1938], however, suggest that the main reason for the rise of B.P. is not an increase of the amount of blood in circulation, but an increase in peripheral resistance as the result of vasoconstriction. Finally, Eichler [1937] and Mügge [1937] maintain that the rise of B.P. is caused by a simultaneous peripheral and heart action. Those authors, again, who suggest the chief factor in producing the rise in B.P. to be an increase in the peripheral resistance do not agree as to the manner in which this peripheral effect arises. Results obtained by perfusion of isolated organs furnish a further source of controversy. According to Lindner [1937], veritol has no influence on the lumen of the vessel. Mügge [1937] found a constriction of the vessel in the perfused rabbit's ear. Biehler [1937] found vasoconstriction in the rabbit's ear and cat's tail.

In order to clear up these points of controversy as to the nature of the action of veritol, a series of experiments were planned. These were designed to study the following points of importance:—

1. Action on B.P.
2. Action on heart.
3. Action on vessels: (*a*) peripheral, and (*b*) central.
4. Other actions.

1. Action on B.P.

Experiments were carried out with anæsthetised decerebrated and decapitated cats. Ether was used for producing anæsthesia. In the

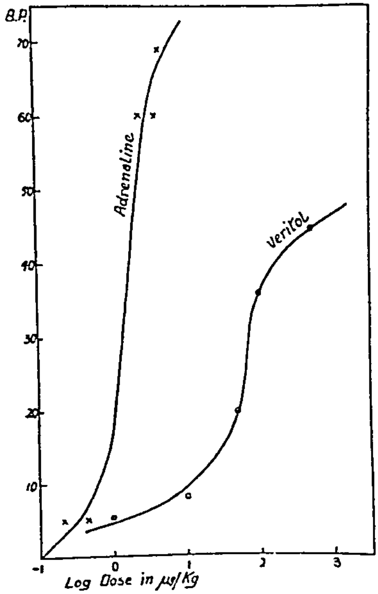

Fig. 1.—Quantitative relation between dosage of veritol and adrenaline and rise of blood-pressure in decerebrated cat (wt. 2·0 kg.).
Abscissa: log. dose in μg./kg. Ordinate: rise of B.P. in mm.

intact animals, chloralose was used at a later stage (100 mg./kg.; 1 per cent. solution intravenously). The B.P. was recorded by means of a mercury manometer in the carotid artery. Various doses of veritol were injected intravenously, and the effects were quantitatively compared with doses of adrenaline.

The following results were obtained:—

(*a*) The ratio between equi-active doses of veritol and adrenaline was found to be 1 : 100 (as far as the extent of the rise of B.P. is concerned).

(*b*) The quantitative relation between log. dose and rise of B.P. is represented by a sigmoid curve (see fig. 1). In the same animal veritol never can produce an effect equal to the maximum obtained with adrenaline.

Fig. 2 shows the relation between dosage and duration of action of

veritol. With doses of 10 μg./kg. or less the durations are similar to those obtained with similar quantities of adrenaline, but whereas 10 μg./kg. adrenaline produced a nearly maximum response, the same dose of veritol produced about one-tenth of this response (*cf.* fig. 1). In this range the relation between log. dosage and duration is approximately linear. With doses of veritol of 100 μg./kg. and over, which produced a nearly maximum response, the duration was approximately

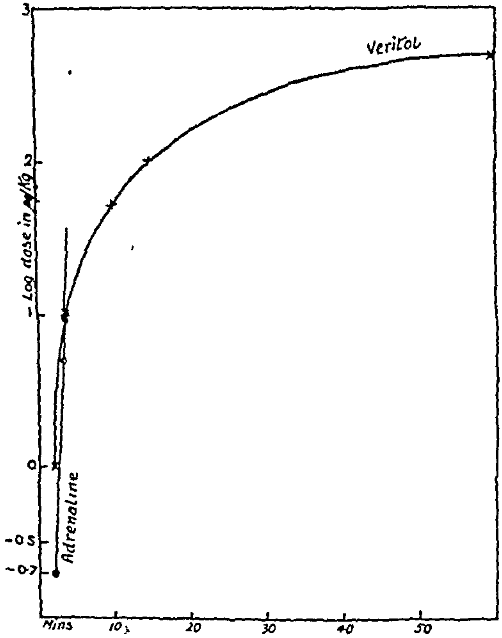

FIG. 2.—Quantitative relation between dosage and duration of action of veritol and adrenaline in decerebrated cat (wt. 2·0 kg.).
Abscissa: duration in min. Ordinate: log. dose in γ/kg.

proportional to the dosage. This type of complex relation between dosage and duration was obtained by Clark and Raventos [1939] with ephedrine. Figs. 1 and 2 together show that when equi-active doses which produce a considerable response are compared, then the duration of action of veritol is much longer than the duration of action of adrenaline.

2. Action on the Heart.

The action on the heart was studied in cats *in situ* by measuring the influence of veritol on:

(*a*) heart rate,
(*b*) minute volume,
(*c*) stroke volume,
(*d*) auricular and ventricular contractions.
(*e*) venous and carotid pressure simultaneously.

(*a*) *The heart rate* was measured in vagotomised cats under chloralose anæsthesia and in decerebrated and decapitated cats under ether anæsthesia. It was found that veritol had practically no influence on the heart rate. Small doses of 10 μg./kg. showed no effect, large doses of 100 μg./kg. and over sometimes produced a slight increase. Adrenaline usually caused a marked increase in heart rate.

(*b*) The *minute volume* was measured by means of a specially designed apparatus (fig. 3). A glass tube of the shape shown in fig. 3 was constructed. The space XY contains a steel ball of a diameter slightly less than that of the internal bore of the tube. The liquid may thus

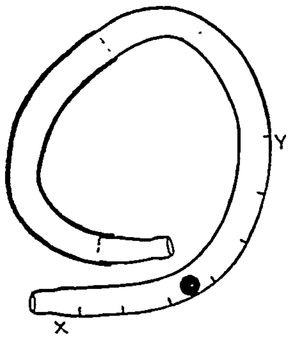

Fig. 3.—Apparatus for measuring the minute volume (described in the text).

flow past the ball, but since the latter offers resistance to the flow, it is forced up into the inclined portion of the tube. As the tangent to any point on the tube continually changes in direction, the ball will come to rest at that point where equilibrium is attained between the hydrodynamic pressure of flow and the resultant gravitational force acting on the ball at that point.

The point of equilibrium for a given rate of flow is determined, on the one hand, by the internal diameter of the tube, and, on the other hand, by the resistance of the ball, which is dependent on its diameter and specific gravity. By direct measurement of the rate of outflow of a stream of blood, passing through the tube, the curve was graduated in c.c. per min. The instrument was fitted in place in the severed vessel as shown, the horizontal portion being directly inserted into the proximal part and the vertical portion being connected *via* a rubber tube to the distal portion. In this way any great displacement of the vessel was avoided. Results may be recorded directly by reading or by photographing the position of the ball. Heparin was used as an anticoagulant and proved to be satisfactory. Instead of a tube with a gradual curve, there may be used a straight tube, the internal diameter of which gradually increases towards the distal end, held at an angle

of about 45°. By altering the angle, the range of the apparatus may be varied.

The minute volume is markedly increased by veritol (fig. 4). This effect is dependent on the size of the dose. As the heart rate is practi-

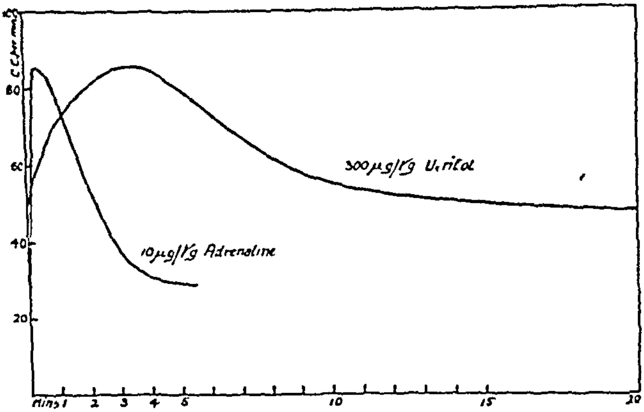

Fig. 4.—Response of minute volume after veritol and adrenaline in vagotomised cat, measured in aorta near its bifurcation.

Abscissa: time in minutes. Ordinate: c.c. per min.

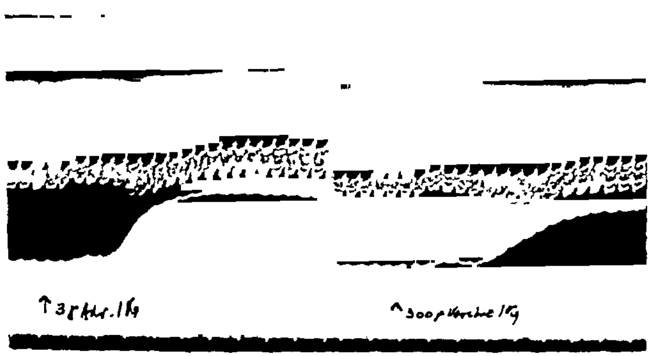

Fig. 5.—Tracing showing from above downwards. (1) Portal vein pressure, (2) stroke volume, (3) carotid B.P. Cat, chloralose anæsthesia. Time 10 secs.

cally unchanged, the increase of the minute volume must be due to an increased stroke volume. The intensity and duration of the effect on the minute volume roughly follows the change in B.P., although the increase of the minute volume in many cases lasted longer than the rise in B.P. (for explanation, see later in perfusion experiments). Adrenaline caused a large but short-lasting increase of the minute volume.

(c) *Stroke volume* was recorded by the heart oncometer. The vagal nerves were cut. Chloralose anæsthesia was used. In many cases this experiment was combined with the measurement of the pressure in the portal veins and carotid artery (see fig. 5).

It was found that the stroke volume was enlarged by veritol, the extent and duration increasing with increasing doses of the drug. The effect was more prolonged than with adrenaline. After adrenaline the portal venous pressure always rose, but only very rarely was this observed with veritol (see fig. 5).

(*d*) *Auricular and ventricular contractions* were measured by Cushny's myocardiograph in normal hearts and in hearts which had been affected with evipan. It was found that both auricular and ventricular contractions increased with veritol, the extent and duration of the effect

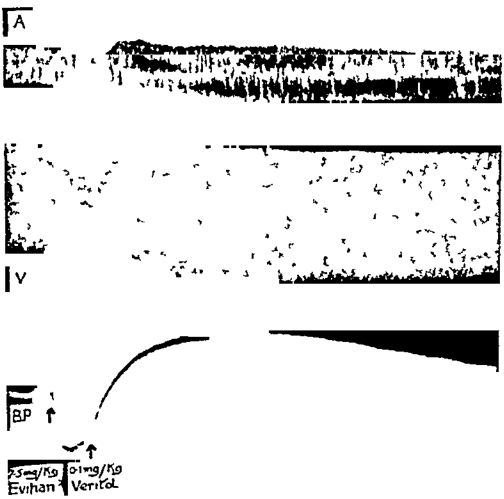

FIG. 6.—Tracing showing action of sodium evipan and veritol on B.P. and contractions of auricle and ventricle in cat.

varying with the dosage. This was true with maximum doses of even 20 mg./kg.

Adrenaline also increased both these movements, the duration, however, being always shorter than with equi-active doses of veritol. When sodium evipan was injected intravenously it caused a fall of blood-pressure and a decrease of auricular and ventricular contractions. Veritol antagonised the action of evipan on the B.P. and heart contractions, as also did adrenaline (see fig. 6).

(*e*) The venous pressure and carotid pressure were examined simultaneously before and after the heart had been affected with evipan. The venous pressure was measured in the jugular vein as near as possible to the junction with the subclavian vein. It was found that the venous pressure was increased after evipan and decreased after veritol (see figs. 7A and 7B).

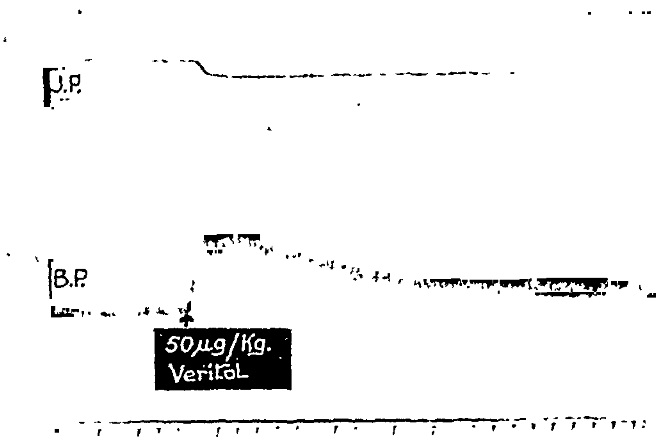

FIG. 7A.—Tracing showing change in the pressure in jugular vein and carotid artery before and after injection of veritol in cat.

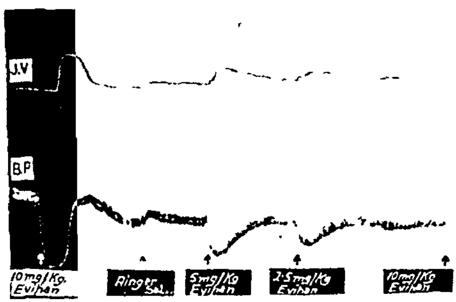

FIG. 7B.—Tracing showing change in the pressure in jugular vein and carotid artery before and after injection of sodium evipan in cat.

3. Action on the Vessels.

(*a*) *Methods.*—In order to study the effect of veritol on blood-vessels, perfusion experiments were carried out on cats in the following manner:—

Oxygenated blood was provided to a perfusion pump (described by Robson and Schild, 1938) by a cannula inserted in the central end of the left femoral artery. The perfusion pump was connected to a cannula in the peripheral end of the right femoral artery. The sciatic and femoral nerves and also the vein of the right leg were dissected out and a thick ligature of copper wire was tied tightly round the limb in order to prevent collateral circulation, leaving free the nerves and vein. This ligature was placed close to the hip joint. Heparin or chlorasol fast pink was used as anticoagulant.

Records of perfusion pressure and carotid blood-pressure were taken

simultaneously. The perfusion pressure was brought to a constant level and after some time drugs were injected either into the general circulation or into the perfusion system close to the cannula. In one experiment the perfusion was carried out with blood (of the same animal) which did not circulate between limb-body-pump and back to the limb, but only flowed from pump to limb and back to pump. The blood was mechanically oxygenated in this experiment.

In all experiments the nerves connecting the limb with the body were intact, which was verified by the injection of cardiazol and evipan. Both drugs, known to act on the central nervous system, showed their

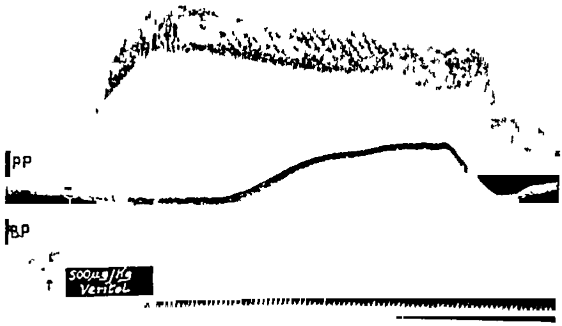

FIG. 8A.—Tracing showing response of carotid pressure (B.P.) and pressure in perfused limb (P.P.) of cat after veritol, without central action. Rise of perfusion pressure due only to local action.

immediate action on the pressure of the isolated limb when the nerves had not been cut.

(*b*) *Results.*—In seven experiments the injection of adrenaline or of veritol into the blood going to the perfused limb caused an immediate vasoconstriction. This response was identical in size and duration before and after cutting the nerves. In order to see whether this local effect could be combined with a central effect of these drugs, veritol and adrenaline were injected into the general circulation. These injections always caused an immediate rise of the carotid B.P. and a rise of the B.P. of the perfused limb occurred after a delay of about 2 minutes, which was due to the time taken to circulate from the body *via* the pump into the limb (fig. 8A). This rise of pressure in the perfused limb was again due solely to a local vasoconstrictor effect, since the amount of perfusing blood was kept constant by the pump. Identical results were obtained after injections into the general circulation, with the nerves intact and with the nerves cut.

The rise of pressure in the perfused limb due to the local action of the drugs was not as great as the rise in the carotid blood-pressure, but if during the pressor response to veritol the minute volume of the pump

was increased from 5 c.c./min. to 9·8 c.c., the rise of pressure in the perfused limb was then of the same order as the rise of pressure in the general circulation.

The experiments on the perfused limb prove that veritol produces vasoconstriction. Furthermore, the effects of veritol on the blood-pressure of the intact animal can be imitated in the perfused limb by administering veritol, and at the same time increasing the rate of perfusion.

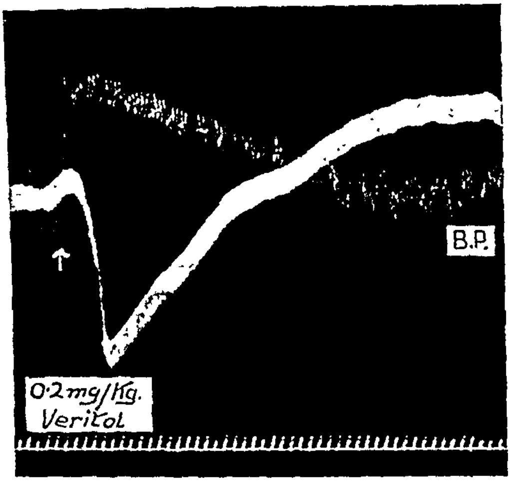

Fig. 8b.—Tracing showing response of carotid pressure and pressure in perfused limb after veritol, with central effect.

The effects produced on the blood-pressure of the intact animal can therefore be accounted for on the supposition that veritol increases the cardiac output and also causes vasoconstriction. The extent to which each contributes to the total effect is variable; the local constrictor effect was on the average one-third of the effect on the B.P. of the whole animal.

In the experiments described, it has been shown that veritol and adrenaline caused only a local action on the vessels. In four other experiments, however, veritol or adrenaline not only had the local vasoconstrictor effect two minutes after the injection into the general circulation which has been described, but these drugs also caused an immediate fall of the perfusion pressure simultaneously with a rise of the carotid blood-pressure (fig. 8b). This fall of perfusion pressure, however, could only be obtained in these experiments so long as the nerves were intact. When the nerves had been cut, an injection into the general circulation caused only the usual immediate rise in the carotid B.P. and the delayed rise in the perfusion pressure. This proves that in these four experiments veritol and adrenaline caused not only a local, but also a central, effect on the blood-vessels.

Finally, in one case there was an immediate rise in the pressure in the perfused limb, which was abolished by nerve section and was therefore due to a central action. In these experiments the vagi were cut and the carotids ligatured. The central effects therefore were not due to a carotid sinus reflex. In a few experiments, where the preparation had been perfused for some hours, the local vasoconstrictor action of veritol was reversed and vasodilatation occurred, although adrenaline still produced vasoconstriction. In a few experiments in which the limb was perfused with Locke's fluid, the same difference was observed. Adrenaline produced a vasoconstriction, but veritol produced a vasodilatation. The local vasoconstrictor action of veritol is therefore only produced in limbs in good condition and perfused with blood.

4. Various Actions.

Experiments were carried out *in vitro* on isolated auricles and ventricles of the frog heart and on the gut of the cat, rat, and rabbit.

In frog's auricles and ventricles veritol in low concentration (10^{-7}) caused increased contraction; in higher concentrations (10^{-4}) decreased contraction. Adrenaline caused a small increase of amplitude at concentrations of 10^{-8}, and a large increase at concentrations of 10^{-6}. The gut *in vitro* proved to be very insensitive to veritol. Inhibition, however, was found in concentrations above 10^{-4}. Adrenaline inhibited the gut at concentrations of 10^{-7} or less.

An adrenaline-like effect of veritol was observed on the nictitating membrane. The contractions, however, lasted much longer than those after adrenaline.

The chief qualitative differences observed between the actions of veritol and of adrenaline were as follows: (*a*) In certain cats small doses of adrenaline produced a fall of blood-pressure, but even in these individuals veritol produced a vasoconstrictor action only; (*b*) adrenaline causes a rise in the portal vein pressure, whilst veritol rarely (once in 12 animals) produces this effect; (*c*) adrenaline causes marked cardiac acceleration, whilst veritol, even in large doses, only produces a slight increase; (*d*) veritol fails to produce vasoconstriction in limbs perfused with Locke's solution or in blood-perfused limbs after prolonged perfusion, whilst adrenaline can produce vasoconstriction under these conditions.

Summary.

1. The rise of blood-pressure produced by veritol is due to two factors: (*a*) vasoconstriction, and (*b*) increased volume output per beat of the heart. Veritol (unlike adrenaline) causes only slight cardiac acceleration.

2. The vasoconstrictor action of veritol can be demonstrated

easily on perfused limbs, provided that blood is used for perfusion and the preparation is in good condition.

3. Veritol sometimes causes a vasodilatation of central origin in perfused limbs.

4. As regards the majority of its effects veritol resembles adrenaline, but its effects are of much longer duration.

5. Doses of veritol as large as 20 mg./kg. did not produce any immediate toxic effects on anæsthetised cats.

I wish to express my thanks to Professor A. J. Clark for his advice and help throughout this investigation. I also wish to thank Mr. N. E. Condon for his advice in designing the stromuhr.

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ACETYLCHOLINE ACTION ON THE PULMONARY VASCULAR BED OF THE DOG AND ITS MODIFICATION BY ADRENALINE AND BY ERGOTOXINE.

By P. Foggie. From the Physiology Department, University of Edinburgh.

(*Received for publication 20th July 1939.*)

In the course of experiments designed to test the value of eserine, ergotoxine and atropine as diagnostic agents for the determination of the nature of pulmonary vasomotor nerves, it was found that both adrenaline and ergotoxine suppressed or reversed the pulmonary arterial pressure rise produced by large doses of acetylcholine. This paper gives an account of experiments which shed some light on the nature of the phenomena.

It is well known that large doses of acetylcholine constrict the pulmonary blood-vessels of the dog [Bennati, Gautrelet and Holpern, 1930; Daly and Euler, 1932; Gaddum and Holtz, 1933; Alcock, Berry and Daly, 1935], an effect which is potentiated by eserine and suppressed by atropine.

In isolated perfused lungs of the dog, small or moderate doses of acetylcholine which cause pulmonary vasodilatation may, after eserine, produce vasoconstriction, which in all probability is due to an increase in the effective concentration of the ester consequent upon the inhibition of the esterase action [Gaddum and Holtz, 1933; Alcock, Berry and Daly, 1935]. The last-named workers, however, obtained results which suggested that acetylcholine may be acting on two different parts of the pulmonary vascular bed, since on rare occasions eserine converted an acetylcholine constrictor to a dilator response. In this connexion Franklin [1932] made the interesting observation that the extrapulmonary arteries of the dog invariably relax and the smaller and larger extrapulmonary veins contract to acetylcholine.

The pulmonary constrictor action of acetylcholine can be obtained in fully nicotinised isolated perfused lungs and is suppressed by atropine [Alcock, Berry and Daly, 1935], and therefore cannot be due to the nicotine-like action of acetylcholine [Dale, 1914] on the pulmonary ganglia. It would appear, therefore, that the pulmonary constriction caused by acetylcholine in isolated perfused lungs of the dog is due to a peripheral mechanism and that any action which adrenaline or ergo-

toxine may have in determining the type of acetylcholine response is of considerable interest.

Methods.

In all experiments isolated lungs of the dog were perfused at constant volume inflow with blood obtained from the same animal. The blood was defibrinated and the lungs ventilated by rhythmical negative pressure variations applied to their outer surface [Alcock, Berry and Daly, 1935]. Records were taken of the pulmonary arterial pressure (P.A.p.) and of the volume of the venous reservoir (V.R.). A rise in the V.R. tracing denotes an increase in venous outflow and a reduction in blood volume of the lung [Daly, 1928].

Results.

The Effect of Adrenaline on the Acetylcholine (A.Ch.) Response.—In one experiment a slight pressor effect of a large dose of A.Ch. was converted

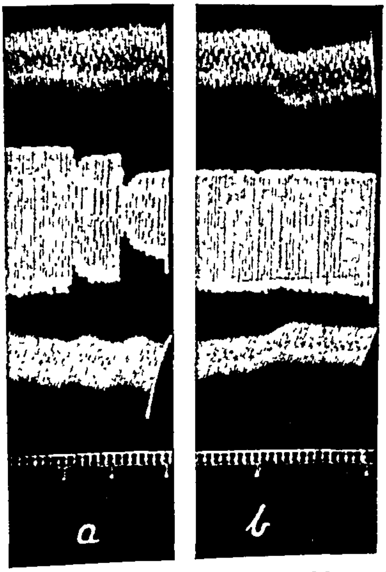

Fig. 1.—Dog, 18·5 kg. Isolated perfused lungs (I.P.L.).
a = successive doses of A.Ch. 500 μg. and 1·0 mg.
b = A.Ch. 1·0 mg.
Between a and b a single injection of adrenaline 200 μg. was given.
P.A.p. = pulmonary arterial pressure; T.A. = tidal air;
V.R. = venous reservoir volume record.

to a depressor by a single injection of adrenaline, and in 4 experiments doses of A.Ch. which were ineffective or produced a slight fall in P.A.p. caused a well-marked fall in P.A.p. after a single injection of adrenaline (fig. 1).

The effect of adrenaline on the A.Ch. response was tested in another way. In seven experiments a series of increasing doses of A.Ch. were injected and the P.A.p. response recorded. In some of these experiments eserine was added to the blood (fig. 2, A). In a further six experiments the same procedure was adopted whilst an adrenaline solution (5 μg./min. to 50 μg./min.) was being steadily infused into the circulation (fig. 2, B). It will be seen from this figure that in the adrenalinised preparations the dilator effect of A.Ch. predominates, whereas in the preparations without adrenaline the response was variable. In some experiments single injections of adrenaline suppressed

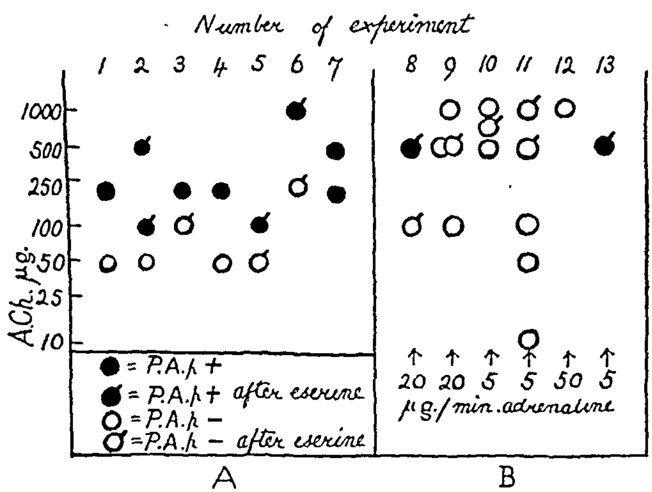

FIG. 2.—Graph showing the effect of adrenaline infusions on the P.A.p. response to A.Ch.

the pressor effect of A.Ch. These observations have not been included in the figure, since a gradual loss of sensitivity of the preparation might have been responsible for the phenomenon.

These results are remarkable in that they are in apparent contradiction to those of Dale and Narayana [1935] and of Ettinger and Hall [1935], who, working on the perfused lungs of the guinea-pig and rabbit, respectively, found that the constrictor action of A.Ch. was potentiated by adrenaline. They concluded that in all probability adrenaline exerted its effect by raising the tonus of the pulmonary blood-vessels, acetylcholine being most effective when the tonus is high. Dale and Narayana [1935], however, recognised that under suitable conditions an increased vessel tonus might promote an A.Ch. dilator response in the guinea-pig, and therefore further work is necessary before the discrepancy between the results reported here and those on the guinea-pig and rabbit can be attributed to a species variation alone.

The Effect of Ergotoxine on the Acetylcholine Response.—The rise in pulmonary arterial pressure produced by large doses of A.Ch. injected into the inflowing blood stream of isolated perfused lungs of the dog is suppressed or reversed by ergotoxine (fig. 3). In 3 experiments the administration of ergotoxine converted the rise to a fall, in one the rise was suppressed, and in another experiment in which the initial effect of A.Ch. was a fall in pressure, this response was not altered after ergotoxine. Moreover, a dose of A.Ch. ineffective on the P.A.p. may, after ergotoxine, become depressor (fig. 4).

During an examination of A.Ch. effects in nicotinised preparations, Alcock, Berry and Daly [1935] observed that ergotoxine converted a well-marked A.Ch. pressor response to a slight depressor (fig. 3 of their paper). This appeared at the time to be so remarkable that no special comment was made. As a result of further investigations there seems no doubt that under suitable conditions ergotoxine suppresses partially or wholly, or reverses the A.Ch. pressor effect in fully nicotinised lung preparations. Out of seven experiments in such preparations, in which large doses of A.Ch. caused a pressor effect, ergotoxine caused partial suppression of the response in one, complete suppression in three, and a reversal in three (fig. 5).

There can at the moment be no absolute certainty that the conversion by ergotoxine of an A.Ch. rise in P.A.p. to a fall is due to the conversion of a contraction response of smooth muscle fibres of the pulmonary vascular bed to one of relaxation, because as we know [Franklin, 1932] A.Ch. may exert a contraction of some of the lung blood-vessels and a relaxation of others, and thus ergotoxine may merely suppress the A.Ch. contractile response and leave unaltered those vessels which normally relax to A.Ch. In this event the A.Ch. fall in P.A.p. after ergotoxine would be due to the unmasking of the normal dilator action of A.Ch. on certain parts of the vascular bed.

Alcock, Berry and Daly [1935] observed that A.Ch. injected into the pulmonary artery at high perfusion pressures tended to cause a smaller vasoconstriction or larger vasodilatation than a similar dose injected at low perfusion pressures. They showed that this phenomenon was due to a greater dilution of the A.Ch. with blood at high perfusion pressures than at low, the smaller concentrations of A.Ch. tending to produce dilatation, the larger constriction. That a similar complication cannot account for the reversal of A.Ch. effects by ergotoxine, which itself produces a rise in P.A.p., is shown by figs. 3 and 5. In these experiments the A.Ch. reversal by ergotoxine took place at the higher perfusion pressure due to ergotoxine, and also at the pressure level prior to the injection of ergotoxine.

Finally, Loewi and Navratil [1926] reported that ergotamine exerts an inhibitory effect on the choline esterase, and in this respect it should render A.Ch. more effective. Since small doses of A.Ch. tend to lower

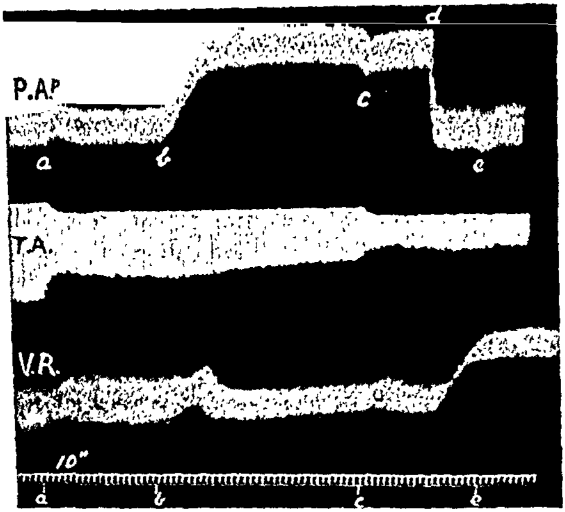

FIG. 3.—Dog, 18·0 kg. I.P.L. Non-eserinised preparation. Reversal of A.Ch. pressor effect by ergotoxine.
a = A.Ch. 500 μg.; b = ergotoxine 3·0 mg.; c = A.Ch. 500 μg.; d = pump inflow diminished; e = A.Ch. 500 μg.

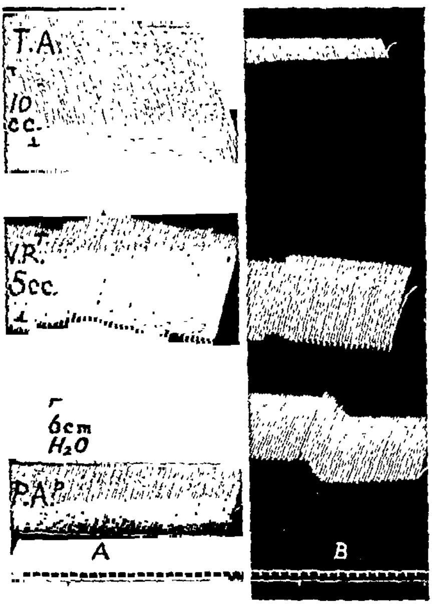

FIG. 4.—Dog, 3·7 kg., ♀. I.P.L.
A = acetylcholine 5·0 μg.
B = acetylcholine 5·0 μg.
Between A and B ergotoxine ethane sulphonate 2·0 mg.

the P.A.p. and large doses to raise it, the action of ergotoxine on the esterase should tend to convert an A.Ch. fall in P.A.p. to a rise, or

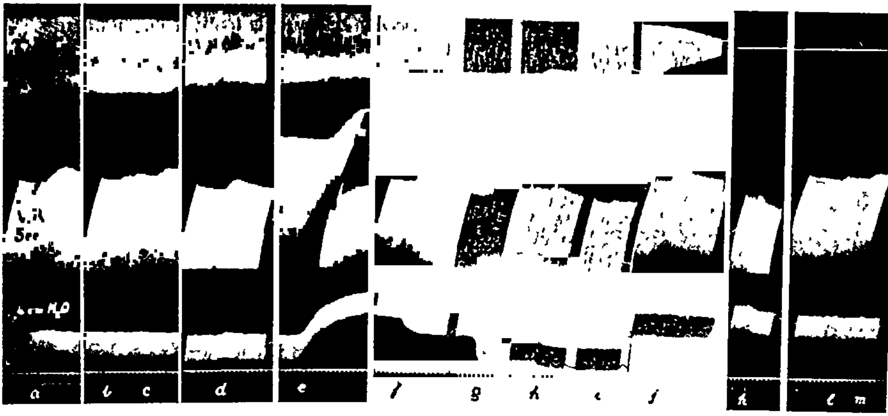

FIG. 5.—Dog, 7·9 kg. I.P.L.

a and *b* = successive doses of nicotine 5·0 mg. in 0·7 c.c. saline.
c = 0·7 c.c. saline (control).
d = *acetylcholine* 50·0 μg.
e = ergotoxine 1·0 mg.: V.R. lever lowered at ×.
f = *acetylcholine* 50·0 μg. in 0·25 c.c. saline.
g = output of pump reduced.
h = *acetylcholine* 50·0 μg. in 0·25 c.c. saline.
i = 0·25 c.c. saline (control).
j = eserine 1·0 mg. (P.A.p. and V.R. levers raised).
k = adrenaline 20·0 μg.
l = nicotine 5·0 mg. in 0·5 c.c. saline.
m = 0·5 c.c. saline (control).
Time = 10 sec.

potentiate a rise. The fact that the effect of ergotoxine on the P.A.p. response to A.Ch. is the reverse of this expectation is all the more significant.

SUMMARY.

In isolated lungs of the dog under negative pressure ventilation and perfused at constant volume inflow with defibrinated blood, it has been found that:

1. In non-nicotinised preparations adrenaline tends to suppress or reverse the rise in pulmonary arterial pressure produced by large doses of acetylcholine.

2. In fully nicotinised preparations, ergotoxine partially or wholly suppresses, or reverses the pulmonary arterial pressure rise produced by large doses of acetylcholine.

I wish to express my thanks to Professor I. de Burgh Daly and to Miss Catherine O. Hebb for helpful advice, and to the Moray Endowment Committee for defraying part of the cost of the research.

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AN EXPERIMENTAL ANALYSIS OF THE ACTION OF ADRENALINE AND HISTAMINE ON DIFFERENT PARTS OF THE PULMONARY VASCULAR BED. By

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IT has been shown in isolated lungs of the dog perfused at constant inflow volume with defibrinated blood and under negative pressure ventilation that adrenaline raises the pulmonary arterial pressure (P.A.p.) and causes a transient increase in venous outflow (V.O.). The precise conditions under which these changes occur, and the precautions which have to be taken to eliminate errors in the interpretation of the results have already been described [Alcock, Berry and Daly, 1935; Daly, 1938]. The adrenaline response described above takes place in the vast majority of lung preparations with doses from 2·0 μg. to 1·0 mg. injected into the stream of inflowing blood in the pulmonary artery tubing, but on occasions we have observed a rise in P.A.p. with a fall in V.O. with larger doses of adrenaline.

The significance to be attached to the adrenaline increase in P.A.p. and V.O. is the problem we propose to discuss with the aid of a description of relevant experiments. The complexity of the problem has been stressed elsewhere [Daly, 1938], and it may be summarised by the statement that in lungs perfused at constant inflow volume the adrenaline rise in P.A.p. may be due to an increase in the resistance of the pulmonary arterioles, capillaries or venules, separately or together; whereas the transient V.O. augmentation leading to a diminution in blood volume of the lungs may also be due to constriction of the arterioles, capillaries or veins squeezing blood from the lungs, or to venous dilatation with consequent passive collapse of the capillaries and drainage of capillary blood into the veins. We have also attempted to determine the site of action of histamine on the pulmonary vascular bed.

I. Simple Perfusion of the Pulmonary Circulation.

Methods.

In the experiments described in this section both lungs of the dog were perfused at constant volume inflow with defibrinated blood and

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ventilated by extrapulmonary negative pressure variations. The venous outflow changes were measured by recording the volume alterations of blood in the venous reservoir; an upward movement of the lever in all V.O. tracings denotes an increasing blood outflow and a diminishing amount of blood in the lungs [Daly, 1928, 1938]. The animals were bled to death from the femoral artery under local anæsthesia. The drugs used were crystalline adrenaline or adrenaline solution with chloretone (P. D. & Co.), histamine acid phosphate (B. D. H.), the dosage being expressed in terms of the base. In all experiments described in this section the injections were made into the pulmonary artery.

Results.

Adrenaline and Ergotoxine.—It has previously been shown [Daly and Euler, 1932; Gaddum and Holtz, 1933] that ergotoxine suppresses or

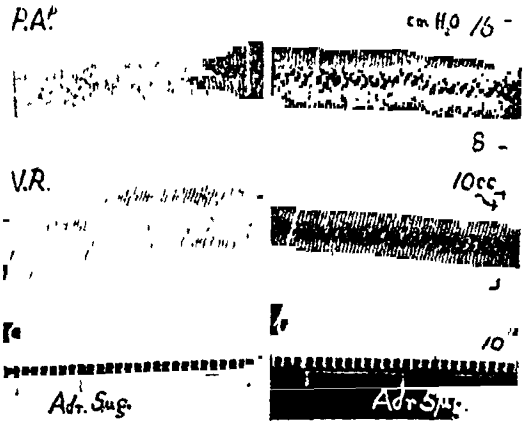


Fig. 1.—Dog, 16·0 kg. I.P.L. The effect of ergotoxine on the adrenaline outflow augmentation. Between *a* and *b*, ergotoxine ethane sulphonate 3·0 mg. P.A.p. = pulmonary arterial pressure. V.R. = venous reservoir volume.

reverses the adrenaline P.A.p. rise in dogs. The adrenaline constricted vessels may be arterioles, capillaries or veins. Hitherto no experimental data has been available regarding the action of adrenaline on the vessels governing the V.O. in ergotoxinised lung preparations. In 35 tests made on 20 such preparations, adrenaline produced the normal outflow augmentation in 2, no outflow response in 26, and a reduction in V.O. in 7 (fig. 1). This suppression or reversal by ergotoxine of the adrenaline outflow increase is in all probability due to the fact that ergotoxine either suppresses or alters an adrenaline constrictor response of some portion of the pulmonary vascular bed to a dilator response. This appears to be a safe assumption, since as far as we are aware evidence has not been produced that ergotoxine either suppresses or

converts an adrenaline dilatation of smooth muscle to an adrenaline constriction. If we are so far correct, it follows that the usual V.O. augmentation observed in perfused isolated lungs is not due to venous dilatation releasing blood stored in the capillaries, but to constriction of the arterioles, capillaries or veins squeezing blood out of the lungs.

That the vascular territory constricted by adrenaline and giving rise to a P.A.p. augmentation differs from that—also constricted by adrenaline—which is responsible for the V.O. increase, is suggested by previous observations in this laboratory. It has been found that cocaine may potentiate the adrenaline effect on the P.A.p. but not that effect upon the V.O. [Daly, Foggie and Ludány, 1937, see their fig. 1]. This we have confirmed. Moreover, adrenaline in small doses may cause a V.O. augmentation without producing any significant alteration in the P.A.p. [Alcock, Berry and Daly, 1935].

These considerations suggested to us that the simplest interpretation of the adrenaline P.A.p. and V.O. increases following the injection of small doses is a constriction of arterioles and venules, the former effect raising the P.A.p., the latter squeezing blood from the lungs (capacity effect).

This conception of adrenaline activity in isolated perfused lungs does not take into account the possibility that the capillaries may play some part in determining the lung responses, and therefore we planned further experiments in the hope of settling this point and for the purpose of testing the truth of our speculations concerning the action of adrenaline on the arterioles and venules (see Section II.).

Histamine.—We have ascertained that the vascular response of the isolated lungs of the dog depends upon the previous history of the preparation and the mechanical conditions of perfusion. Since our experiments may throw some light upon the discrepancies in the literature regarding histamine action, we will describe them briefly.

Alcock, Berry and Daly [1935] reported that histamine in all doses caused a transient reduction in venous outflow. During a further investigation we were unable to confirm this result, for histamine augmented just as frequently as it reduced the outflow. Moreover, small doses of histamine tended to augment the outflow, whereas the larger doses gave either an increase or decrease (fig. 2, Table I.). On searching for possible causes of this discrepancy we discovered two outstanding differences in the experimental procedure adopted by the two groups of investigators. In the earlier series the animals were bled to death under chloroform and ether anæsthesia, or under local anæsthesia; in the later series all the animals were bled to death under local anæsthesia. Further, in the interval of time between the two series of experiments the perfusion apparatus was redesigned, but as far as could be judged the only significant alteration made was in the level of the venous reservoir in relation to the left auricle.

In the earlier series the blood pressure in the left auricle was lower than in the later series owing to the venous reservoir receiving the blood being at a lower level. It seemed therefore worth while to test whether

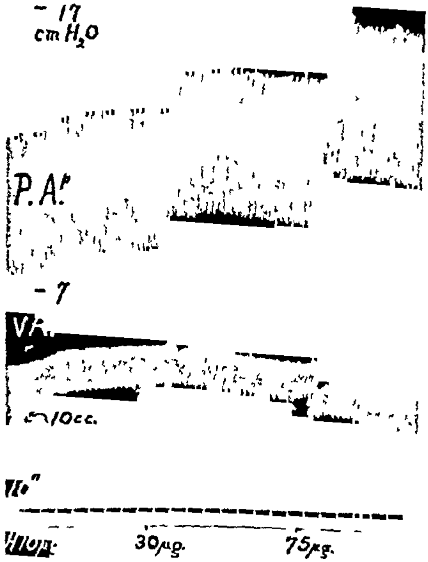


Fig. 2.—Dog, ♀, 12·0 kg. I.P.L. Effect of increasing doses of histamine on the venous outflow. Three successive doses of histamine 10, 30, and 75 µg.

Table I.—Effect of Histamine on Isolated Perfused Lungs (Dog) prepared from Animals bled under Local Anæsthesia.

Dose, µg.	P.A.p.		V.O.			T.A.		No. of tests.
	o	+	o	+	−	o	−	
0·5 to 10·0	7	7	4	9	1	4	10	14
11·0 to 50·0	3	12	1	4	10	2	13	15
51 and more	3	15	4	6	8	5	13	18

Minimal effective dose on T.A. = 1·0 µg.; on P.A.p. = 5·0 µg.; on V.O. = 5·0 µg.
P.A.p. = pulmonary arterial pressure; V.O. = venous outflow; T.A. = tidal air.
+ = increased; − = decreased.

a volatile anæsthetic or the height of the left auricular pressure had any influence in determining the nature of the pulmonary vascular response to histamine.

A perfused preparation was made from an animal bled under local anæsthesia and histamine injected before and during ventilation with an air-ether mixture, and again after ventilation with air alone. Fig. 3

shows that the venous outflow following histamine injections tends to show a greater fall in lungs ventilated with an air-ether mixture than

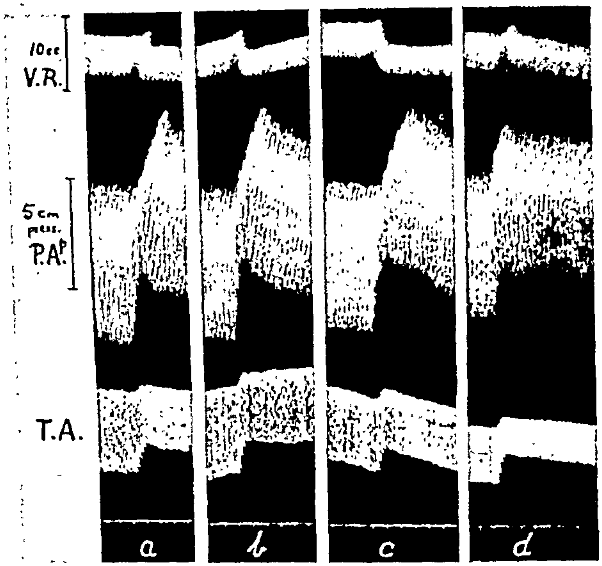


FIG. 3.—Dog, ♂, 13·5 kg. I.P.L. Effect of histamine, 15·0 μg. injected at *a*, *b*, *c*, and *d*. *a* = before ether; *b* = after 10 min. of ether inhalation; *c* = immediately after ether removal; *d* = 30 min. later. T.A. = tidal air.

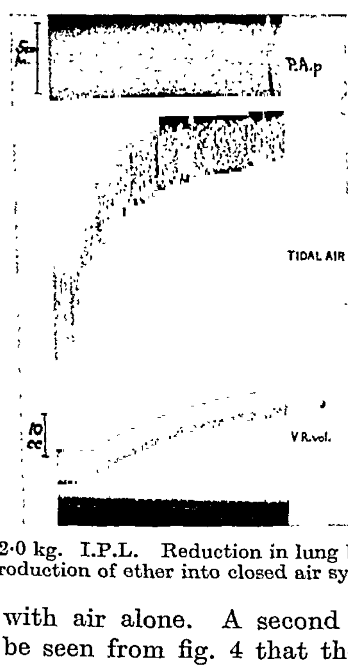


FIG. 4.—Dog, ♀, 12·0 kg. I.P.L. Reduction in lung blood volume due to introduction of ether into closed air system.

in lungs ventilated with air alone. A second experiment confirmed this result. It will be seen from fig. 4 that the effect of introducing

In the earlier series the blood pressure in the left auricle was lower than in the later series owing to the venous reservoir receiving the blood being at a lower level. It seemed therefore worth while to test whether

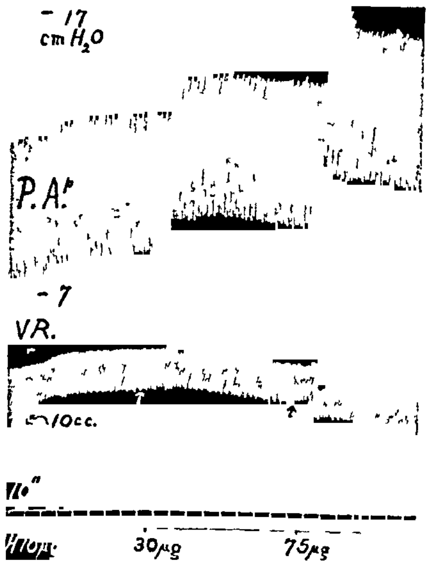


Fig 2.—Dog, ♀, 12·0 kg. I P.L. Effect of increasing doses of histamine on the venous outflow. Three successive doses of histamine 10, 30, and 75 μg.

Table I.—Effect of Histamine on Isolated Perfused Lungs (Dog) prepared from Animals bled under Local Anæsthesia.

Dose, μg.	P A p.		V.O.			T.A.		No. of tests.
	o	+	o	+	–	o	–	
0 5 to 10 0	7	7	4	9	1	4	10	14
11 0 to 50 0	3	12	1	4	10	2	13	15
51 and more	3	15	4	6	8	5	13	18

Minimal effective dose on T.A. = 1 0 μg.; on P.A.p = 5·0 μg ; on V.O = 5 0 μg. P.A.p. = pulmonary arterial pressure; V.O. = venous outflow; T A. = tidal air. + = increased; – = decreased.

a volatile anæsthetic or the height of the left auricular pressure had any influence in determining the nature of the pulmonary vascular response to histamine.

A perfused preparation was made from an animal bled under local anæsthesia and histamine injected before and during ventilation with an air-ether mixture, and again after ventilation with air alone. Fig. 3

cating bed of capillaries which drains into the pulmonary veins [Küttner, 1878; Miller, 1937; Zuckerkandl, 1881, 1883]. It occurred to us that we might take advantage of this vascular arrangement for the admission of drugs to the arterial side alone, or to the venous side alone of the pulmonary vascular bed and thus be able to select the site of drug action. Let us consider the diagram shown in fig. 6 of

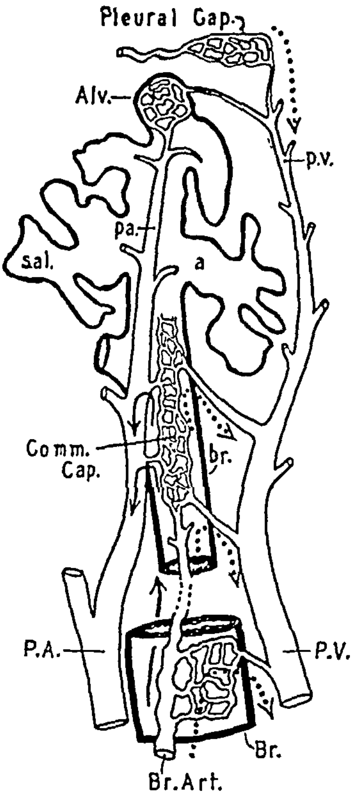


FIG. 6.—Diagram to illustrate the route taken by drug solutions injected into the pulmonary vascular bed by way of the bronchial arteries of isolated lungs perfused in the normal and reverse direction. See text.

the blood supply to a primary lobule which is a modification of that published by Miller [1937]. When the lungs are perfused through the pulmonary artery, *without* simultaneous perfusion of the bronchial circulation, the blood passes to the pulmonary arterioles, the alveolar capillaries and to the capillaries of the respiratory bronchioles, thence to the pulmonary veins. If a drug solution is injected into the bronchial artery, it should reach the capillaries of the bronchi as well as the respiratory bronchiole capillaries and be carried by the perfusion blood stream into the pulmonary veins without having reached the pulmonary arterioles. Owing to a limited region of the visceral pleura at the lung

ether into the intrapulmonary tidal air recorder space is to empty the lungs of blood.

With regard to the effect of left auricular pressure on the histamine response: the pressure was raised by means of a screw-clip placed round the outflow rubber tubing. The clip was screwed up until the increased resistance to outflow caused an appreciable increase in lung blood volume. Injections of histamine at the higher auricular

Fig. 5.—Dog, ♂, 13·5 kg. I.P.L. Effect of histamine 50 μg. injected at *a*, *b*, and *c*; *a* and *c* at low venous pressure; *b* at high venous pressure.

pressures tended to suppress the diminution in outflow which occurred at the lower pressures (fig. 5).

It is interesting to note that the histamine diminution in venous outflow can be most readily demonstrated under the two conditions (a low pulmonary venous pressure and ether administration) which reduce the initial quantity of blood in the lungs. We have not attempted to determine the relative importance of these conditions governing the type of histamine outflow response, but we gain the impression that the anæsthetic is the dominant factor.

II. Perfusion of the Lungs in the Normal and Reverse Direction with Injection of Drugs into the Pulmonary Circulation by way of the Bronchial Arteries.

The bronchial arteries supply the respiratory tree as far as the respiratory bronchioles, and in this region break up into a communi-

tions injected with Indian ink from the bronchial artery alone a few scattered injected areas were seen over the cut surface of some of the lung lobes. Each covered only a few square millimetres and were streakily black, not uniformly black, in appearance. Some areas surrounded small bronchioles, but others appeared to be in the parenchyma. In one experiment Indian ink was observed in the superior azygos vein.

Histological examination of two further lung preparations in which bronchial arterial injections of carmine-gelatine and Indian ink solutions respectively were carried out at the end of perfusions lasting several hours gave us additional information. In the lungs injected with carmine-gelatine, the mixture was confined to the bronchial arteries, bronchial veins, pulmonary veins, and a few alveolar capillaries adjacent to the bronchioles; the remaining alveolar capillaries showed no trace of carmine-gelatine. If it can be assumed that the injection of drug solutions into the bronchial artery followed the same vascular route as that filled by the carmine-gelatine, then the drug action would be confined as anticipated to the limits marked out by the dotted arrows in fig. 6. In the lungs injected with Indian ink solution, the ink particles completely filled the bronchial arteries, the bronchial veins and some small pulmonary veins, as well as the capillaries belonging to a few of the alveoli adjacent to the respiratory bronchioles. These vessels appeared as solid black streaks. The vast majority of pulmonary capillaries contained no Indian ink particles at all, but here and there isolated capillaries contained a few scattered particles. In a few sections small groups of adjacent alveoli stretching longitudinally across the field appeared well filled with Indian ink and serial sections showed that these were alveoli, adjacent to a respiratory bronchiole, which had been sectioned in a plane parallel to but not including the respiratory bronchiole. Taking into account this experiment and the others already described, it is considered that under conditions of pulmonary perfusion the action of drug solutions injected into the bronchial artery will be confined for the most part, or in some experiments almost exclusively, to the vascular territory in the region of the respiratory tree and to those larger pulmonary vessels which drain it.

Methods.

In the majority of experiments the method used was similar to that described by Daly [1938] for perfusion of monkey's lungs. All the experiments were performed on dogs bled to death from the femoral artery under local anæsthesia. For perfusion of the lungs in the reverse direction the output of the pump was permanently connected to the cannula in the left auricle and the outflowing blood from the pulmonary artery collected in the reservoir, or, the rubber tubing

hilus being supplied by the bronchial artery [Miller, 1906, 1907], it is possible that a portion of the drug solution will reach the pleural veins which empty into the pulmonary veins. The route of the injected drug solution is shown by the dotted arrows. It should be stressed that the bronchial arteries are only being used as a channel for carrying the solution to the pulmonary venules and that they do not contain *circulating* blood. Although the bronchial arteries as well as the pulmonary venules may be responsive to the drug injected, the pulmonary venule response alone will affect the P.A.p. because it is only these vessels, with which perhaps should be included the relatively small number of capillaries on the venous side of the communicating bed, which contain circulating blood. Likewise, alterations in the outflow will be for the most part determined by the venules, although it is conceivable that if constriction of the bronchial arterioles and capillaries occurred, it might squeeze out sufficient blood into the pulmonary veins to cause a small increase in venous outflow.

By perfusing the lungs in the reverse direction and injecting the drug solutions into the bronchial artery, it should be possible to confine the action of the drug almost exclusively to the pulmonary artery and arterioles. The route of the injected solution under these conditions is shown by the solid arrows (fig. 6).

Injections of Indian ink solutions diluted with approximately an equal volume of saline either into the pulmonary arterial tubing or into the R. posterior bronchial artery during perfusion of the lungs gave us some indication of the correctness of these premises. In two experiments the Indian ink injected into the bronchial artery reached the cannula in the left auricle in a shorter time than when injected into the pulmonary arterial inflowing blood stream. In one of these the time taken for the Indian ink to pass from the pulmonary artery to the left auricle was 13 secs., and from bronchial artery to left auricle 8·7 secs. (mean of 3 observations). Following the injections into the pulmonary artery, the ink appeared in the left auricular cannula well mixed with blood, but in the case of the bronchial arterial injections, streaks of ink were apparent in the blood in the cannula. Thus the pathway in the axial blood stream from bronchial artery to left auricle is shorter than from pulmonary artery to left auricle.

In four further experiments Indian ink was injected into the bronchial artery only. In two of these the bronchial tubes of the right lung but not the left were deeply stained; in the other two experiments the bronchial tubes of the left lung were also stained but to a lesser extent than those of the right lung. These results are in conformity with those of Alcock, Berry, Daly and Narayana [1936], who injected barium sulphate gelatine mixtures into the main R. posterior bronchial artery, and from subsequent radiographic studies found that in some experiments the L. lung remained uninjected. In all the lung prepara-

volume of fluid injected, and thus subsequent changes in the record due to the action of the drug were more easily detected.

When drugs are injected into the pulmonary circulation by way of the bronchial artery the pulmonary venules will be the first vessels to be reached by the drug in lungs perfused in the normal direction, and the pulmonary arterioles will be the first reached during "reverse" perfusions. The initial responses of the inflow pressure and outflow volume will be due to the respective reactivity of these vascular territories, but if sufficient of the drug recirculates, a secondary series of responses may take place due to the passage of the drug from the pulmonary arteries to the capillaries and veins during normal perfusion, and from the pulmonary veins to the capillaries and arteries during "reverse" perfusion. In order to make certain that the responses obtained were due to the reactivity of the vessels immediately receiving the drug solutions, we used in some of our experiments a method to prevent recirculation effects.

The usual single blood reservoir was replaced by two separate vessels (fig. 7, R, R′) in communication with each other by means of wide bore glass tubing (C.T.) dipping to the bottom of each reservoir and joined together by a short piece of wide bore rubber tubing at the point marked X to permit the application of a clamp outside the respiratory chamber for the purpose of separating the blood in the two reservoirs. Blood volume changes in the reservoirs were recorded in the usual manner by putting them in communication with a volume recorder. The pump finger stall (F.S.) and the valve chamber (V.) are shown in the figure, but the lungs and arrangements for keeping the blood at 37° C., as well as the orientation of the apparatus with the respiratory chamber are omitted. These details may be obtained from an earlier paper [Daly, 1938].

When testing the effect of drugs injected into the pulmonary circulation by way of the bronchial artery the following procedure was adopted. Simultaneously with a control saline injection, the communicating tube (C.T.) was clamped. As a result it was found that no significant change occurred in the inflow pressure, but in nearly every case a change in level of the venous recorder lever took place, in part due to the volume of injected fluid and in part due to the separation of the reservoirs (fig. 8). The change in level due to separation of the reservoirs took place immediately the tube C.T. was clamped. Since a similar level change occurred when the lungs were replaced by a rubber bag, the cause was due to mechanical and not to physiological effects. In all probability it would have disappeared if the resistance of the communicating tube had been negligible; that this tube possessed some resistance to the flow of blood from R′ to R was shown by the slightly lower blood level of R from which the pump drew blood as compared with that of R′ which received the blood from the lungs.

carrying the blood to and from the lungs was arranged so that, by clamping off alternative channels, perfusion could be changed over from the normal to the reverse direction at will.

For the introduction of solutions into the bronchial vascular system the main R. posterior bronchial artery or the parent intercostal artery was cannulated as described by Berry, Brailsford and Daly [1931]. Similar quantities of solution were injected at each test, and control

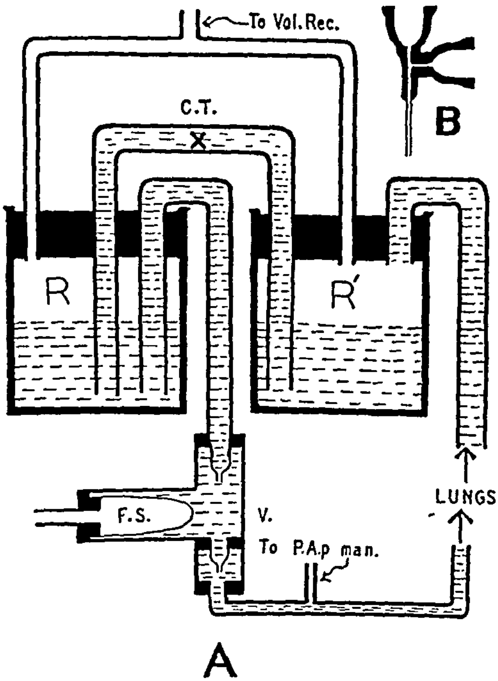


FIG. 7.—A. Arrangement of venous reservoir connexions to prevent recirculation effects of injected drugs. See text.

B. Diagram of needle with double head for the administration of drug solution and saline "wash-in" to be performed as one continuous injection.

saline injections made at intervals. The drug solution was injected from a syringe into a long hypodermic needle, which passed through the respiratory chamber to the bronchial arterial cannula. This injection was then immediately followed up by a saline injection from a second syringe in order to wash the cannula free of the drug. This procedure was greatly facilitated by fitting the hypodermic needle with a double head (fig. 7, B) to accommodate the two syringes. The advantage of this method lay in that the administration of the drug and the washing in with saline were performed as one continuous injection, so that there was only one elevation of the volume recorder lever due to the

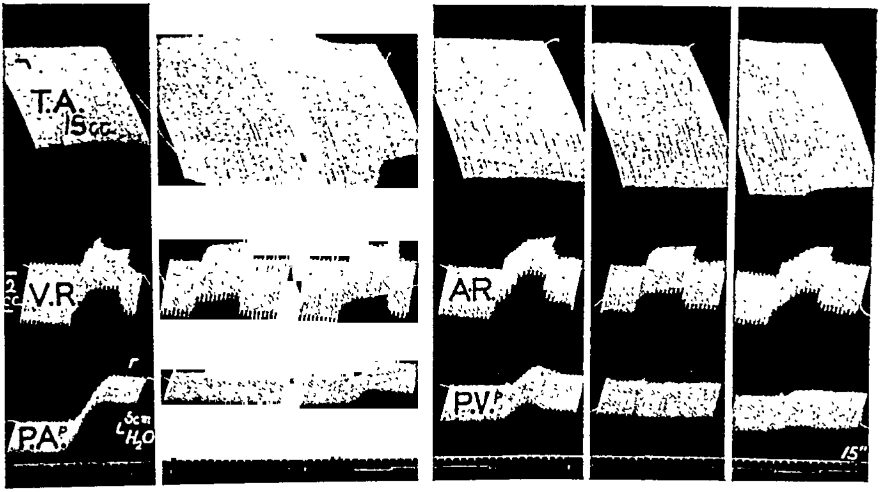
Having recognised the shift of the venous volume tracing on clamping the communicating tube—and its return to the initial level after release of the clamp—it only became necessary to perform such controls at intervals throughout the experiment and compare the responses obtained with those due to drug injections. In making the comparison of the V.R. tracings, attention should be drawn to three points, namely: the difference from one tracing to another of (1) the levels of the upper peaks of the excursion, (2) the levels of the lower peaks, and (3) the change in mean level of the tracing subsequent to putting the reservoirs in communication. During occlusion of the communicating tube between R and R′ the blood level in R fell and that in R′ rose. The output of the pump and capacity of the reservoirs were such that 45–120 seconds were available for test before it was necessary to put the reservoirs once more into communication with one another and thus prevent complete emptying of R.

Results.

The action of drugs on the inflow pressure and on volume outflow in isolated lungs perfused in the normal and the reverse direction.

Adrenaline.—We confirmed the previous result from this laboratory [Alcock, Berry and Daly, 1935] that adrenaline injected into the pulmonary artery of isolated lungs (dog) perfused in the normal direction with defibrinated blood almost invariably causes a rise in P.A.p. and a transient increase in V.O. (Table II. (1) and fig. 8, obs. 1). We found, somewhat to our astonishment, that adrenaline injected into the inflowing blood of lungs perfused in the reverse direction led to a raised inflow pressure—that is, a raised pulmonary venous pressure (P.V.p.) and also to a transient increase in the outflow from the pulmonary artery (Table II. (3) and fig. 8, obs. 4). Thus adrenaline augments the inflow pressure and the volume outflow whether perfusion is carried out in the normal or in the reverse direction. These effects occurred using either the single or separated reservoir method, and in preparations perfused throughout in the normal or in the reverse direction as well as in those arranged so that the direction of perfusion could be changed at will. Before attempting to interpret these results, we propose to discuss the effect of injecting adrenaline into the pulmonary vascular bed by way of the bronchial arteries.

It was found that adrenaline introduced into the pulmonary vascular bed by the bronchial arteries augmented the inflow pressure and the volume outflow during both normal and reverse perfusions (Table II. (2), (4), figs. 9 and 10). Thus if we are correct in our assumption that adrenaline injected into the bronchial artery acts almost exclusively on the pulmonary venous vessels during normal perfusion, and almost exclusively on the pulmonary arterial vessels during reverse perfusion,

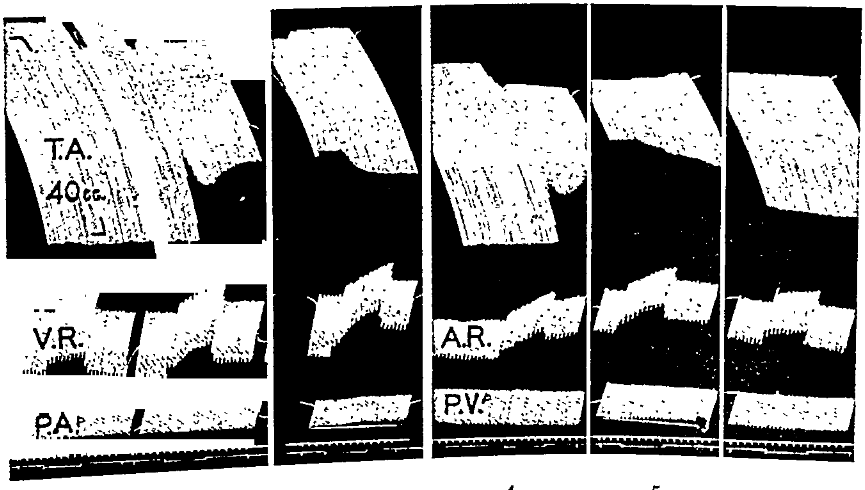


1	2	3	4	5	6
Adrenaline 25 μg.	Control Saline.	Histamine 15 μg.	Adrenaline 10 μg.	Control Saline.	Histamine 5 μg.

Normal Perfusion. Reverse Perfusion.

All Pulmonary Arterial Injections.

FIG. 8.



1	2	3	4	5	6
Control Saline.	Histamine 30 μg.	Adrenaline 25 μg.	Histamine 30 μg.	Adrenaline 25 μg.	Control Saline.

Normal Perfusion. Reverse Perfusion.

All Bronchial Arterial Injections.

FIG. 9.

FIGS. 8 and 9.—Reservoirs separated and injections made at beginning of signal; reservoirs put into communication at end of signal. A.R. = arterial reservoir; P.V.p. = pulmonary venous pressure.

bronchial arterial injections of adrenaline during reverse perfusion is due to constriction of the pulmonary arterial tree.

Returning now to the group of experiments in which the pulmonary vascular bed was perfused in the normal or reverse direction and adrenaline injected into the *inflowing blood-stream*, the rise in inflow pressure and the transient augmentation in outflow which occurred becomes more readily understood. These responses are presumably due in part to constriction of both arteries and veins raising the resistance to inflow and squeezing blood from the lungs—effects which occur equally well in whichever direction the lungs are perfused.

Table II. shows that increases in inflow pressure and in outflow volume nearly always accompany one another when adrenaline is

Table II.—Effect of Adrenaline and Histamine Injections into the Bronchial and Pulmonary Arteries during Normal and Reverse Perfusion of Lungs.

	P.A.p.			V.O.			P.V.p.			A.O.			No. of Obs.
	o	+	−	o	+	−	o	+	−	o	+	−	
Adrenaline.													
(1) P.A. inj. N.d.	0	30	1	3	28	0		..			..		31
(2) B.A. inj. N.d.	10	12	0	2	19	1		..			..		22
(3) P.V. inj. R.d.		..			..		3	17	0	0	20	0	20
(4) B.A. inj. R.d.		..			..		4	3	1	0	8	0	8
Histamine.													
(5) P.A. inj. N.d.	4	11	0	4	11	0		..			..		15
(6) B.A. inj. N.d.	5	6	0	2	9	0		..			..		11
(7) P.V. inj. R.d.		..			..		1	5	1	4	3	0	7
(8) B.A. inj. R.d.		..			..		2	3	0	2	3	0	5

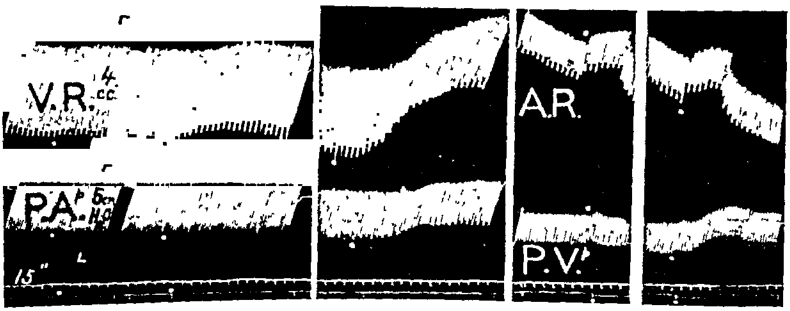
A.O. = arterial outflow; B.A. = bronchial arterial; inj. = injection; N.d. = normal direction of perfusion; p. = pressure; P.A. = pulmonary arterial; P.V. = pulmonary venous; R.d. = reverse perfusion; V.O. = venous outflow. o = o change; + = increased; − = decreased.

injected into the pulmonary vessels either during normal or reverse perfusion. On the other hand the bronchial arterial injections of adrenaline with perfusion in either direction appear less effective in raising the inflow pressure than in augmenting the outflow. We interpret this result as indicating that the larger arteries and veins of the pulmonary vascular bed which are chiefly influenced by bronchial arterial injections of adrenaline do not always constrict sufficiently to produce a resistance as well as a capacity effect. When, however, adrenaline is injected into the pulmonary artery or veins, it reaches

the rise in inflow pressure must be due chiefly to venous constriction in the former circumstances and to arterial constriction in the latter.

In order to produce an adrenaline response of the pulmonary vascular bed by injections into the bronchial artery, somewhat large doses have to be used, due to the fact that only a portion of the dose reaches the lungs; dye injections showed that the remainder escapes into the œsophageal and other arterial vessels which freely anastomose with twigs of the bronchial artery at the root of the lungs.

Further light is thrown upon the nature of these responses by the following considerations. Provided that all sizes of blood-vessels of the pulmonary venous tree—when acted upon exclusively by adrenaline injected into the *bronchial arteries* during normal perfusion—respond



Control Saline. | Adrenaline 50 μg. | Adrenaline 500 μg. | Control Saline. | Adrenaline 50 μg.

Normal Perfusion. Reverse Perfusion.

All Bronchial Arterial Injections.

Fig. 10.

in a like manner, a venous constriction is the only type of response which will give a concomitant rise in inflow pressure (resistance effect) and a transient augmentation in outflow (capacity effect); the latter effect being due to the squeezing of blood from the veins. It is clear that the augmented outflow cannot be due to venous dilatation releasing blood from the capillaries, for if this were the nature of the response, the inflow pressure would show, if anything, a fall. The capacity effect would be expected to occur without a significant change in vessel resistance when small doses of adrenaline, producing only a mild venous constriction, are injected; whereas with larger doses the stronger venous constriction would be expected to superimpose a resistance increase upon the capacity effect. This order of events, of which fig. 10 is an example, has been observed on a number of occasions. A similar line of argument leads us to the conclusion that the augmentation in inflow pressure and outflow volume produced by

shows that successive doses of 10 μg. and 1·0 mg. of adrenaline reduce the lung blood volume by 10 and 30 c.c. respectively in a perfused preparation made from a dog of 22 kg. body weight. The total blood capacity of the arterial and venous vessels down to twigs of approximately 200 μ would not greatly exceed 50 c.c. if the measurements made by Daly [1938] can be accepted. If constriction of the capillaries was not in any way responsible for the lung blood volume reduction, 1·0 mg. of adrenaline must have diminished the volume of the arterial and venous vessels to 40 per cent. of the initial value, a not impossible

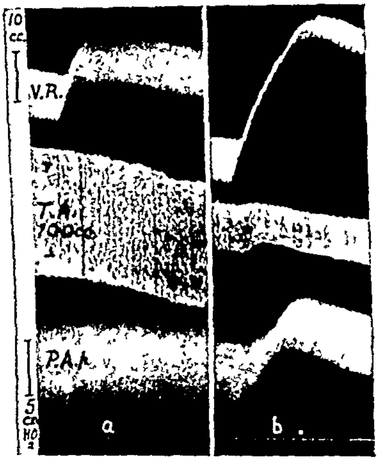


FIG. 11.—Dog, ♂, 22·0 kg. Isolated lungs perfused in the *normal* direction. a = adrenaline, 10 μg.; b = adrenaline, 1·0 mg. Both injections made into the inflowing blood-stream.

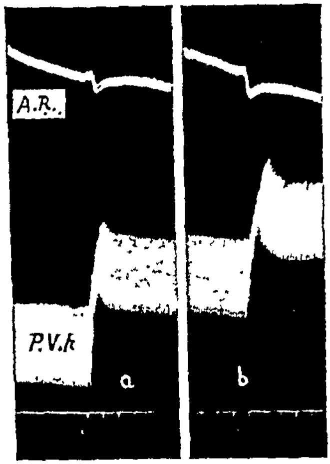


FIG. 12.—Dog, ♂, 14·0 kg. Isolated lungs perfused in the *reverse* direction. a = adrenaline, 50 μg.; b = adrenaline, 250 μg. Both injections made into the inflowing blood-stream. Time = 30 secs.

quantitative effect but an unlikely one in view of the relatively small rise in P.A.p. It could be argued that adrenaline caused venous dilatation and released blood from the capillaries, but the experiments reported in a previous section of this paper lend no support to this view.

We have attempted to tackle the problem in another manner. It is well known that static expansion of the lungs by a negative pressure applied to their outer surface increases the blood volume of the lungs —at any rate in part—by dilatation of the capillaries. If therefore the adrenaline responses of the P.A.p. and V.O. in collapsed lungs were quantitatively similar to those in expanded lungs, it would be reasonable to assume that the capillaries play no part in the production of the responses. Fig. 13 illustrates what we have found in several experiments, namely, that lung expansion not only lessens the effect of adrenaline in augmenting the venous outflow but also diminishes its

not only the larger vessels but those of small calibre which increase their resistance to blood flow however slight the constriction.

It has already been mentioned that adrenaline injected into the bronchial arteries will first reach the capillary bed common to both bronchial and pulmonary circulations in the region of the respiratory bronchioles. Although this common capillary area must be very small, it is possible that the adrenaline inflow pressure rise and outflow augmentation due to bronchial arterial injections may be in small part due to responsiveness of these capillaries. If so, the pressure and outflow changes indicate that capillary constriction of this area causes a resistance increase and capacity diminution, thus potentiating the effect of similar changes in the arteries and veins in reverse and normal perfusions respectively, or that the capillary response if dilator is insufficient to counterbalance the augmentation of inflow pressure and outflow due to constriction of the arteries and veins.

We are well aware that an objection to our hypothesis of adrenaline action on the pulmonary vascular bed can be raised on the score that a constriction of the blood-vessels governing the outflow should lead to a diminished venous outflow due to capillary engorgement which, with very large doses of adrenaline, should preponderate over the augmented outflow caused by the "capacity" effect. The rarity in our experience of a diminished venous outflow response, even when large doses of adrenaline are injected into lungs perfused in the normal direction, suggested that the capillaries might be fully dilated in such preparations, an idea which was not rendered less significant by our experimental records which revealed that the larger doses of adrenaline were always injected towards the termination of the experiment. In order to test the validity of this supposition, large doses of adrenaline were injected into the inflowing blood-stream of lungs freshly perfused in the normal or in the reverse directions. In the former preparations we obtained no definite evidence that a diminished outflow was more likely to occur with the larger adrenaline doses (up to 1·0 mg.) than with the smaller (fig. 11). On the other hand, in reverse perfusion experiments the larger doses in one experiment tended to diminish the outflow (fig. 12), a result which suggests that the vessels governing the outflow in reverse perfusion preparations, *i.e.* the arteries and arterioles, are capable of a stronger constriction than those governing the outflow during normal perfusions, *i.e.* the veins and venules.

Adrenaline and Pulmonary Capillary Response.—Although all our results can be explained on the assumption that adrenaline constricts both arterial and venous vessels in the lungs, we have on occasion obtained such a large transient outflow augmentation and reduction in lung blood volume following adrenaline injections during normal perfusion that it seems unlikely that the arterial and venous vessels alone could by constriction squeeze out the quantity measured. Fig. 11

excursion on the venous reservoir volume tracing in all probability means that the capillaries and/or veins resist expansion to negative pressure, therefore one or both of these territories constrict to adrenaline. If the respiratory excursions are due to variations in the volume of capillary blood, and the capillaries are not constricted by adrenaline, then the diminished respiratory excursion following adrenaline injections must be due to constriction of the veins because the total blood volume of the lungs is reduced. If, on the other hand, the capillaries constrict to adrenaline, this alone will account for the diminution in respiratory excursion. It might be argued, however, that the diminished respiratory excursion of the venous reservoir tracing following adrenaline injections is caused by a maximal dilatation of the veins *or* capillaries, and that collapse of the lungs cannot lead to collapse of the capillaries or veins because they are held in the dilated position. In this event either the veins must dilate whilst the capillaries constrict, or the veins constrict whilst the capillaries dilate; both cannot dilate because the diminished respiratory excursion is accompanied by a reduction in the total blood volume of the lungs. This means that if adrenaline causes active capillary dilatation, a concomitant venous constriction must squeeze out more blood from the lungs than the capillaries take up. Conversely, if adrenaline dilates the veins, the capillaries must actively constrict or passively collapse, letting out more blood than the veins take up. Now, if the capillaries passively collapse because of a diminished venous resistance, the respiratory excursions would increase owing to the greater potential range of capillary calibre change, and thus, if venous dilatation is associated with diminished excursions of the venous reservoir tracing, active capillary constriction must take place. It seems that from all viewpoints the diminished respiratory excursion can only mean that adrenaline constricts either capillaries or veins or both.

The Latent Period of the Inflow Pressure and Outflow Responses.—It might be expected that a knowledge of the time of onset of the inflow pressure and outflow changes following drug injections might help in determining their site of action in the pulmonary vascular bed. When adrenaline or histamine solutions are injected into the inflowing pulmonary blood-stream or into the bronchial arteries during normal or reverse perfusion, careful measurements reveal that the inflow pressure response generally precedes by 2–10 secs. or occurs simultaneously with the outflow augmentation; less frequently the outflow change occurs first. This statement refers to lung preparations perfused with blood at 100–150 c.c./min. Larger perfusion flows than these render the measurement of the total latent period (from injection to first appearance of the response) uncertain. If the inflow pressure or the outflow change occurs alone, then the total latent period is approximately the same as when both responses are present. From

pressor action. When comparing the effects of a drug on the lungs in the collapsed and expanded condition it must be borne in mind that the blood volume of the lungs is greater when the lungs are expanded, and therefore the final dilution of the drug reaching the tissues will be greater when injections are made into the pulmonary artery of expanded lungs. In some experiments, in order to counteract this effect, we introduced the adrenaline into venous reservoirs of the perfusion circulation, taking precautions to keep the reservoir blood volume constant [Alcock, Berry and Daly, 1935]. In this way the concentra-

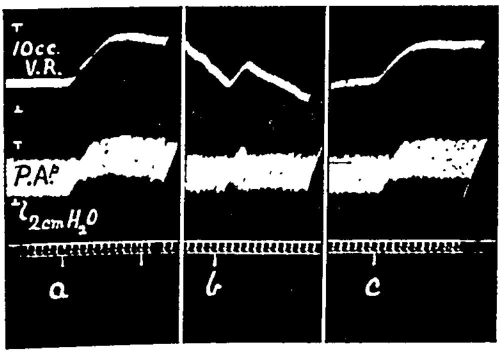
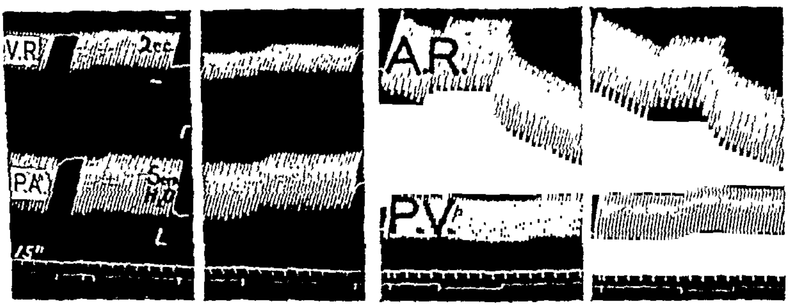


FIG. 13.—Dog, ♂, 16·0 kg. Isolated lungs perfused in the normal direction. a and c = effect of adrenaline, 20 μg. on collapsed lungs; b = adrenaline, 20 μg. on lungs kept expanded by a negative pressure applied to their outer surface. Time = 30 secs.

tion of adrenaline in the blood entering the lungs, whether collapsed or expanded, was the same. Since this modification did not materially affect the result it is concluded that adrenaline constricts those pulmonary blood-vessels which negative pressure expansion dilates—whether these are capillaries alone or arterioles and venules in addition, we are unable to say. In this connexion we have found that the local application of adrenaline to the visceral pleura blanches the lung surface; this effect, however, may be interpreted as being due to arteriole, venule or capillary constriction, for in all probability the colour of the lung is determined by the blood content of all three types of blood-vessel.

The Significance of Respiratory Excursions on the Venous Reservoir Tracing.—It will be noticed in the figures that when adrenaline has any action on the respiratory excursions of the venous reservoir tracing, it is in the direction of a diminution. The familiar to and fro oscillations of blood in the venous outflow tubing of perfused lungs under *negative* pressure ventilation are due to the greater blood capacity of the capillaries and extrapulmonary veins of the lungs when expanded. The intrapulmonary veins may also take part. A diminished to and fro oscillation, following adrenaline injections, reflected as a smaller

Histamine.—Injections of histamine into the pulmonary circulation during forward or reverse perfusion augment the inflow pressure and the outflow (Table II.); in one experiment histamine caused a slight fall in inflow pressure during reverse perfusion. The bronchial arterial injections of histamine also augment both the inflow pressure and the outflow during normal and reverse perfusions. Figs. 9 and 14 show that these two responses may occur independently of one another. These results accord with the view that histamine does on occasion constrict the pulmonary arteries and veins. The dependence of the histamine outflow response upon experimental conditions which we



Control Saline. — Histamine 25 μg. — Control Saline. — Histamine 30 μg.

Normal Perfusion. — Reverse Perfusion.

All Bronchial Arterial Injections.

FIG. 14.—Dog. Effect of histamine injected into the pulmonary vascular bed by way of the bronchial arteries.

have not been able to determine (pp. 23–26) precludes any definite statement as to the action of histamine on the pulmonary vascular bed as a whole, and no doubt largely depends upon the existence of capillary tone.

Discussion and Conclusions.

It has been shown that adrenaline injected into the inflowing bloodstream of lungs perfused in the normal or reverse direction augments both the inflow pressure and volume outflow. The resulting diminution in lung blood volume may amount to as much as 30 c.c. in large dogs, and this represents approximately 60 per cent. of the blood in the lung arteries and veins, or 20 per cent. of the total volume of blood in the lungs [Daly, 1938]. This release of lung blood by adrenaline in lungs perfused at constant volume inflow may be due to arterial, venous or capillary constriction squeezing blood from the lungs or to venous dilatation releasing blood from the capillaries.

The experiments on lungs perfused in the normal and reverse direction with injections of adrenaline made into the bronchial arteries

the examination of a large number of such responses we conclude that their total latent periods can give no certain indication as to the site of action of the drug. This conclusion has been reached firstly because the periods show great variability in any given experiment, and secondly because we consider it probable that the total latent periods not only depend upon the distance of the reactive blood-vessels from the point of injection but also upon the latent period of the response of the vessel itself.

The foregoing remarks relate to a comparison of the latent period of the inflow pressure with that of the volume outflow response. With

Table III.—Action of Adrenaline in Normal and Reverse Perfusions of the Lung.

	Adrenaline Dose, μg.	IN.p. mm.H_2O.	O.R. c.c.	Latent period (sec.).		Perfusion direction.
				IN.p.	O.R.	
1	200	+3·0	+4·9	5·0	4·5	R
	200	+4·5	+4·9	2·7	2·5	N
	200	+2·5	+3·6	5·0	5·0	R
	200	+3·5	+2·6	2·5	4·0	N
	20	+2·25	+5·5	2·5	2·5	N
	20	0·00	+2·0	..	6·0	R
2	40	+2·5	+4·6	4·7	5·0	R
	40	+2·5	+4·6	3·0	4·0	N
3	100	+3·0	+5·0	4·0	3·5	R
	100	+3·0	+4·6	2·0	2·5	N
4	100	+2·5	+1·6	1·5	2·0	N
	100	+1·5	+1·0	4·5	4·0	R

N = normal; R = reverse direction of perfusion; IN.p. = inflow pressure; O.R. = outflow reservoir volume change.

regard to these latent periods in lungs perfused alternately in the normal and reverse directions we observed that both were longer in reverse than in normal perfusions (Table III.). One explanation of this result might be that if the capacity of the pulmonary venous system were greater than that of the pulmonary arterial, the dilution by the blood of adrenaline injected during reverse perfusion would be greater than during normal perfusion and the vascular response correspondingly less rapid and weaker. It has been found, however, that the capacity of the venous system is, if anything, slightly the smaller [Daly, 1938], and therefore this interpretation fails. At the present time we are unable to throw light upon the phenomenon.

Summary.

In isolated dog's lungs under negative pressure ventilation and perfused at constant volume inflow with defibrinated blood—

1. The increase in pulmonary arterial pressure and the augmentation in venous outflow produced by adrenaline are suppressed or reversed by ergotoxine.

2. Evidence is produced which favours the view that the blood-vessels responsible for the adrenaline rise in pulmonary pressure differ from those which cause the increase in venous outflow.

3. Histamine in small doses tends to increase and in large doses to diminish the venous outflow. The venous outflow response to histamine is also determined by other experimental conditions; the presence of ether in the lungs and a low venous pressure favour a histamine diminution in outflow.

Experiments have been reported which suggest that, by injection of drugs into the pulmonary vascular bed by way of the bronchial arteries, it is possible to confine the site of drug action chiefly or almost exclusively to the pulmonary veins in lungs perfused in the normal direction, and in all probability chiefly or almost exclusively to the pulmonary arteries in lungs perfused in the reverse direction. Using this method, results have been obtained which indicate that adrenaline in small doses constricts both pulmonary arteries and veins, and in large doses probably constricts the pulmonary capillaries.

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indicate that both arteries and veins constrict to adrenaline, increasing their resistance and squeezing out the blood within the vessels. With the exception of a vasodilator response to the first dose of adrenaline which occurs on rare occasions [see also Schlesinger, 1931, and Alcock, Berry and Daly, 1935], we have obtained no evidence that adrenaline exerts any constant vascular action on dog's lungs perfused with defibrinated blood other than constriction, and we are forced therefore to the conclusion that large doses of adrenaline in these preparations cause constriction of all parts of the pulmonary vascular bed, including the capillaries, and that small doses cause arterial and venous constriction only.

The rare occurrence of adrenaline vasodilatation in freshly perfused lungs of the dog and of *Macacus rhesus* [Daly, 1938] assumes greater importance in the light of recent work by Petrovskaia [1939]. She has shown in this laboratory that in the perfused lungs of the pig a fall in P.A.p. is the usual response to adrenaline in the early stages of perfusion, but if the injections are continued the response becomes diphasic and finally pressor. After ergotoxine, adrenaline once more causes a fall in P.A.p. Thus the depressor action of adrenaline lasts longer in isolated lung preparations of the pig than in those of the dog or of *Macacus rhesus*. How far this difference is due to the longer maintenance of tone in the pig's pulmonary vessel as compared with the other animal species, or to conditions governing the modification of adrenaline by enzymatic activity, must at the moment be a matter of speculation.

Our results are in agreement with those of other workers who have found that adrenaline almost invariably causes vasoconstriction in isolated perfused lungs of the dog or when acting upon isolated pulmonary artery preparations [Wiggers, 1921; Daly, 1933]; moreover, the location of adrenaline action on the pulmonary veins is in general harmony with the results of Franklin [1932]. This investigator found that adrenaline usually contracts the extrapulmonary veins, and gives very small responses, or none at all, of the intrapulmonary veins, the relaxations balancing in number the contractions. Gaddum and Holtz [1933] in some of their experiments perfused dog's lungs at constant inflow volume, and in 5 out of 10 experiments observed a rise in P.A.p. and a V.O. augmentation; under these circumstances the effect on the volume of the lung was inevitably a fall. They expressed no opinion as to whether the outflow increase was due to dilatation of the pulmonary veins letting the blood flow out, or constriction of the capillaries forcing it out. Our interpretation of their results based on those described in this paper would be in favour of venous and possibly capillary constriction squeezing blood from the lungs.

OBSERVATIONS RELATING TO CARDIAC HYPERTROPHY IN THE RABBIT: ITS PRODUCTION BY ARTERIO-VENOUS ANASTOMOSIS.

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IN any investigation concerning the physiology or pathology of hypertrophied cardiac muscle,[3] it is essential that the characteristics of the hypertrophy which can be produced by various experimental procedures should be known in some detail.

Hypertrophy of the heart can be produced experimentally by creating various defects in the circulatory system. It has been produced, for instance, by aortic stenosis and regurgitation [Rosenbach, 1878], and by stenosis of the pulmonary artery [Reid, 1924; Holman and Beck, 1926]. It is said by some to develop after removal of the buffer nerves [Koch, Mies, and Nordman, 1927; Kremer, Wright, and Scharff, 1924; Boyd and McCullagh, 1938]. Arterio-venous anastomosis has also been shown to lead to cardiac enlargement [Holman, 1924]. Of the experimental methods available the anastomosis of an artery such as the carotid into the jugular vein would appear to be the most convenient. The initial operation can be made without opening the chest and a dilated hypertrophied heart obtained. At any moment the anastomosis can be readily closed by a subsequent simple operation, when the dilatation would presumably quickly disappear and the hypertrophy remain, at any rate for a time. There is, however, some doubt as to the degree of hypertrophy which can be obtained by this method. Shipley, Shipley, and Wearn [1937], using the rabbit, obtained the same degree of hypertrophy in two animals as they did with the other methods they employed, such as aortic regurgitation and pulmonary stenosis. Hermann [1928], using dogs, thought that a relatively high grade of cardiac hypertrophy could be produced by carotid jugular anastomosis and that there is a relatively greater right ventricular hypertrophy. Holman [1937] comes to the conclusion that the cardiac hypertrophy in dogs in the presence of large fistulæ is only slight or moderate, and that the great enlargement

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[3] The criterion for cardiac hypertrophy adopted in this paper is an increase in the weight of the heart to the carcase weight of the animal.

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ligated distal to the opening, an "end to end" anastomosis being formed. The operation was performed under nembutal anæsthesia with aseptic precautions. Young animals of both sexes of 1700–2000 g. have been used throughout, and have been obtained from a variety of breeds through ordinary commercial sources.

If the operation is successful, a continuous murmur is heard over the wound and in a day or so a palpable thrill develops. Within a few days closure of the anastomosis by compression leads to a definite slowing of the heart [Holman, 1937]. A low-pitched continuous murmur, definite thrill, and considerable slowing of the heart upon compression of the anastomosis is generally associated with a large leak. A murmur having a high-pitch quality is heard when a small leak is present. The animal appears to suffer no inconvenience from the leak,[1] recovers from the operation quickly, eats well and steadily gains weight. As time goes on the dilated vein can be readily felt beneath the skin. With a "side to side" anastomosis the eye on the operated side exhibits exopthalmos, and appears congested. Later this condition becomes accentuated and the eye continuously weeps. In addition, at the end of about three months the respirations of the animal become slow and embarrassed. Both these effects appear to be due to the high venous pressure in the distal veins on the operated side, for they disappear completely when the anastomosis is removed. In order to avoid these symptoms the "end to end" anastomosis was made. In such circumstances the animal does not appear abnormal in any way.

The animals were kept for three months. As this stage under urethane anæsthesia observations were made upon the arterial and venous pressures; the heart was washed out and fixed *in situ*, and the size of the leak estimated.

Method of Fixing Hearts.

The animal was anæsthetised with urethane and ether, and under artificial respiration the sternum was split and the heart exposed. A cannula connected to a saline perfusion bottle with a pressure of 30–40 cm. of water was tied into the tip of auricular appendage. In a few experiments a long cannula was inserted with the external jugular vein, so that the opening lay at the junction of the superior vena cava with the right auricle. After ligating the descending aorta and inferior vena cava, the left carotid artery was sectioned, and warm saline allowed to run into the right auricle till the heart was free from blood. Formol saline was then injected and the heart fixed in a dilated condition. The heart was then removed by cutting the large vessels close to their attachment to the heart, and was placed in formol saline for 24 hours and after this in water for 12 hours. The heart was then trimmed in the following manner. The pericardial reflection from the back of the left auricle

[1] In a series of 52 animals in which a leak has been established, two have developed cardiac failure with œdema or ascites. All animals were fed upon a mixture of beet pulp, bran, and clover hay daily, with green food once a week [Mitchell and Pleasance, 1927].

of hearts seen is due mainly to dilatation. In the face of these different opinions it seemed desirable to determine more accurately the character of the hypertrophy which is produced by arterio-venous anastomosis, using a larger number of animals than has hitherto been reported upon. It was decided to use the rabbit, as it has a relatively constant heart weight to carcase weight ratio, and is easy to handle and to keep for long periods of time under standard conditions. The cat was not used, for although the heart weight to carcase weight ratio is relatively constant, it is difficult to handle and keep in good condition in large numbers. The dog was decided against, owing to the considerable difference in the heart weight to carcase weight ratio of the different breeds. There is evidence to suggest that the degree of the hypertrophy produced depends not only on the size of the leak between the artery and the vein, but also upon its position relative to the heart, anastomoses near the heart leading to a greater degree of hypertrophy. As a high degree of hypertrophy was desired, it was decided to anastomose the right carotid artery to the right external jugular vein in the neck. The observations reported in this paper detail the character of the hypertrophy which is produced in the rabbit's heart when these two vessels are anastomosed. At the same time certain information regarding the size of the leak and the arterial and venous pressures is included.

Operative Procedure.

In the beginning the carotid artery was intubated into the jugular vein, the opening of the cut artery inside the vein pointing towards the heart. The method is very simple from the operative point of view and gives good initial leaks, but it is valueless for chronic experiments owing to the fact, observed by other workers, that the cut end of the artery closes naturally in about 5–19 days in a high percentage of animals and the leak in consequence is closed.

The method which was then chosen was to join the artery and vein by a side to side anastomosis. The operative procedure adopted was similar to that already reported by other workers [Holman, 1937], a cut of about 1 centimetre being made in the two vessels. Owing to the small size of the artery the sewing is best performed with dissecting binoculars; very fine curved needles and the finest silk being used. The method does not produce leaks which remain patent in all animals, but with practice a very high percentage of successes can be relied upon.

In all animals the carotid artery was tied distal to the anastomosis. This ensures that the pressure in the carotid sinus on the operated side undergoes no changes as the leak develops, or subsequently if the anastomosis is tied off. The vein was on some occasions left open throughout its course, an anastomosis being produced which has been termed "side to side" in this paper. On other occasions the vein was

has the appearance of a dilated heart. The pericardium contains the normal amount of fluid and there is no hydrothorax. There is no undue amount of fluid in the abdomen and all the viscera appear normal.

In 41 animals in which the operation was performed the leak was never established or sealed up within a very few days. These were used as a control series and were kept for three months before the various heart weights were obtained. The distribution of the various heart weight to carcase weight ratio met with in this series is shown in fig. 1.

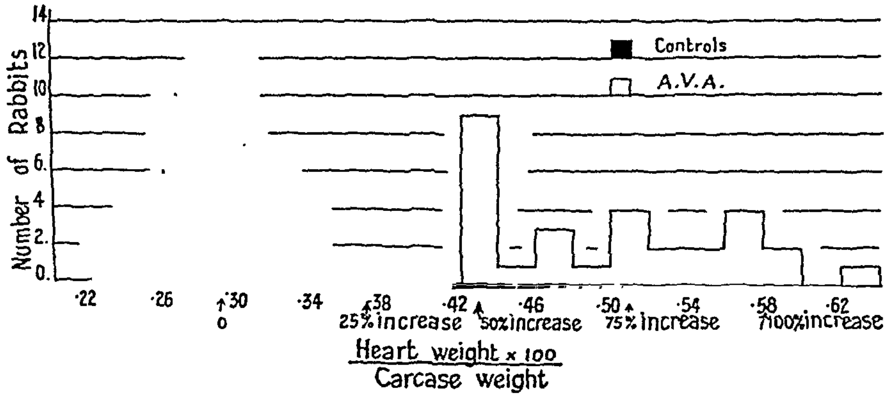

FIG. 1.—Distribution of heart weight × 100 to carcase weight ratios in (1) control rabbits, and (2) those in which the anastomosis had been patent for three months.

In 37 animals the ratio fell within a range extending from ·22 to ·34 with a peak at ·28 to ·30. Four animals had higher values. They were animals in which, for some reason unknown, the weight definitely fell before they were killed. They exemplify the well-known fact that the heart does not immediately lose weight if the body weight falls. For this reason they have been discarded, and only animals have been reported upon in this paper in which the weight either was steadily increasing or was constant.

In fig. 1 the same ratios are also plotted of 29 animals in which the leak had been patent for three months. The ratios found are considerably greater than in the control series. If the average of the controls series is taken at ·29, it will be seen that the increase in heart weight is of the order of 50 p.c. to 100 p.c. or more.

In fig. 2, A the ratios of the auricular weight × 1000 to carcase weight are plotted of the two series. In the controls, the ratios vary from ·20 to ·45 with a high peak of ·29, while in those with a leak it is distributed between ·45 and ·112. From this figure it is seen that increases in auricular weights of as much as 300 p.c. may be encountered, but that is more frequently between 100 p.c. and 200 p.c.

Owing to this very considerable increase in auricular weight, the

and the great vessels with its contained fat was removed, care being taken to avoid removing part of the membranous wall of the auricle itself. The vessels entering the auricle were cut off flush with the wall. The aorta and pulmonary artery were separated and cut off about the site of the semilunar valves. Adherent fluid was then removed by squeezing the heart in a sponge cloth, and the heart weighed. The auricles were next dissected off with scissors following the auriculo-ventricular sulcus around the heart, and separating them from the great vessels. They were then detached by dividing the intra-auricular septum at its junction with the intra-ventricular septum, rough dried and weighed. The two ventricles were also rough dried and weighed. The ventricular mass was then sliced horizontally into three pieces, each containing a piece of each ventricle. The outer wall of the right ventricle was cut away in each slice at its junctions with the septum and left ventricle. The piece containing the vessels was cut by separating the aorta and pulmonary artery and finding a line of cleavage extending a short way down the septum; the right ventricular wall being then separated off so as to include the opening of the pulmonary artery intact. The slices which comprise the left ventricle and septum and the right ventricular wall were then rough dried and weighed. In small hearts such as the rabbit's the division of the septum into right and left portions is difficult, and in view of the data of Hasenfeld and Romberg [1897] for normal and hypertrophied rabbits' hearts, and of Herman [1926] for the dog's heart obtained by the method of ventricular separation, one-third of the weight of the right ventricle was added to its observed weight and an equal amount subtracted from the left ventricle, to obtain corrected values for the two ventricles. From these weights the left ventricular to right ventricular ratio was calculated. In addition, the weight of each auricle was determined by cutting the right and left auricle away from the inter-auricular septum and weighing these three portions separately. One-half of the weight of the septum was added to each auricular weight and the final values taken as the weights of the right and left auricle. This enabled the ratio of the left to right auricular weight to be determined, and by adding the weights to those of the left and right ventricles respectively, the ratio of the weight of the left side to the right side of the heart.

As the heart weight related more closely to carcase weight than to body weight, owing to the considerable and variable weight of the gut, the animal was weighed just before giving the anæsthetic, and at the end of the experiment the stomach and intestines were removed and weighed. This weight was subtracted from the weight of the whole animal and the carcase weight thus obtained.

Results.

In an animal in which the anastomosis has been patent for three months the arterio-venous anastomosis exhibits the usual changes which have been frequently reported [Holman, 1937]. The artery is dilated, frequently almost back to the aorta, and may reach a diameter of 3 mm. or more. The vein is very dilated, extreme dilatation extending in the "side to side" anastomosis about 2–3 cm. distally to the anastomosis and centrally to the entrance of the vein into the thoracic cavity. In the "end to end" anastomosis the vein central to the anastomosis is extremely dilated. No change in the superior vena cava has been noted. On opening the chest the heart appears to be enlarged, and

weight ratios are usually associated with low left heart to right ratio, indicating a relatively greater hypertrophy on the right side, but this is not invariably found.

The results tabulated are from animals of both sexes and of body weights varying from 2000–2600 g. at death in which the leak has been patent for three months. Hypertrophy of such a degree and character is, however, produced well before this time, for it has been found in several animals which have been killed 4 weeks after the operation.

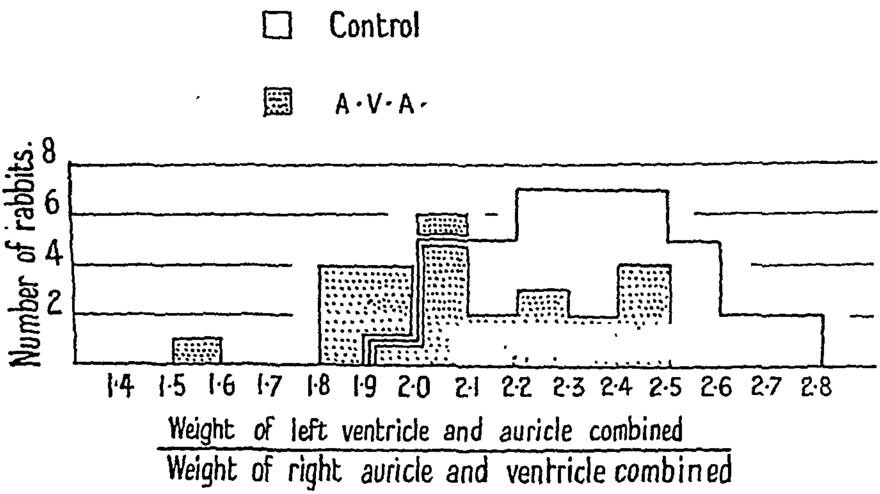

FIG. 3.—Distribution of left ventricular to right ventricular weight ratios in (1) control rabbits, and (2) those in which the anastomosis had been patent for three months.

No difference in the degree of character of the hypertrophy could be detected in the two sexes, or whether the anastomosis was of the "side to side" or "end to end" variety.

In conclusion, it can be stated that if an anterio-venous anastomosis is established between the carotid artery and jugular vein, the heart hypertrophies, on occasions, doubling its weight. The hypertrophy is of such an order that the auricles may weigh three to four times, but the ventricles rarely exceed twice, the average normal weights. The hypertrophy affects all chambers, but there is a tendency for the right to develop a relatively greater hypertrophy than the left heart.

Histological Findings.

As no special methods were adopted to prevent shrinkage due to the fixative, no attempt was made to measure the size of the fibres. This has been done by Shipley, Shipley, and Wearn [1936]. Ordinary histological examination showed that in the hypertrophied auricles an increase in the fibre diameter could be readily appreciated. The myofibrils in the hypertrophied fibres appeared to be coarse and

ratio of the combined ventricular weights × 100 to the carcase weight is plotted in fig. 2, B. The range of the controls is from ·18 to ·32, while in those with leaks it is from ·34 to ·52, so that the increase in ventricular weight is usually between 50 p.c. and 100 p.c. The degree of hypertrophy brought about by the leak tends to be relatively greater in the auricle than in the ventricles.

The ratio of the left auricular weight to the right auricular weight in the control series was extremely variable. In some animals both the

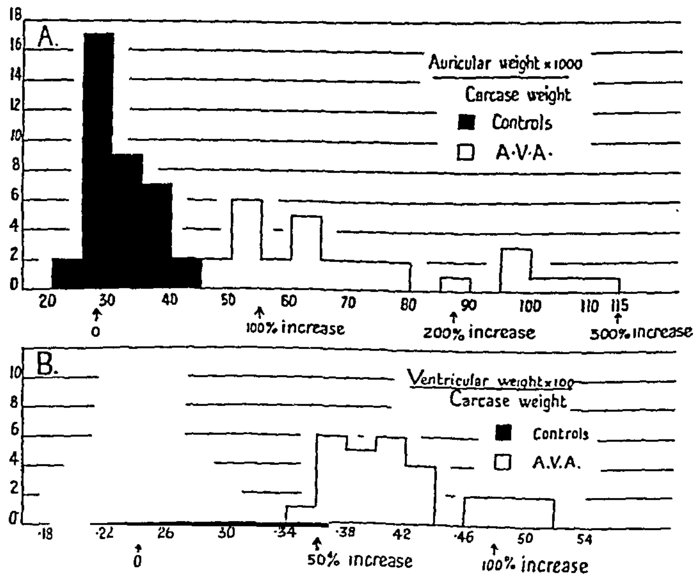

Fig. 2.—Distribution of (A) auricular weight × 1000 to carcase weight, and (B) ventricular weight × 100 to carcase weight of (1) control rabbits, and (2) those in which the anastomosis had been patent for three months.

auricles had much the same weight; in others the left auricles weighed twice as much as the right, the majority lying between these extremes. In the animals with anastomoses this relationship appeared to be generally maintained, both auricles taking an equal share in the hypertrophy.

The ratio of weights of the left auricle and ventricle combined with that of the right auricle and ventricle of both series are plotted in fig. 3. The result indicates that, through and through, the hypertrophy is relatively a little greater in the right side of the heart than in the left, but that a considerable number of hearts have a ratio which is well within the normal limits. The hypertrophy, therefore, is not confined to one side of the heart but in the main is general, with a slight tendency for a relatively greater hypertrophy in the right side. If these ratios are compared with their respective heart weight to carcase weight ratios, the hearts with the higher heart weight to carcase

is not a strict one. This may be due to the different biological response of the hearts in the different animals. It may equally be that the method used in estimating the leaks does not give a real measure of the circulatory defect imposed upon the animal.

Venous Pressures.

At the final operation, the venous pressure relative to the level of the great veins in the chest was measured by connecting a saline

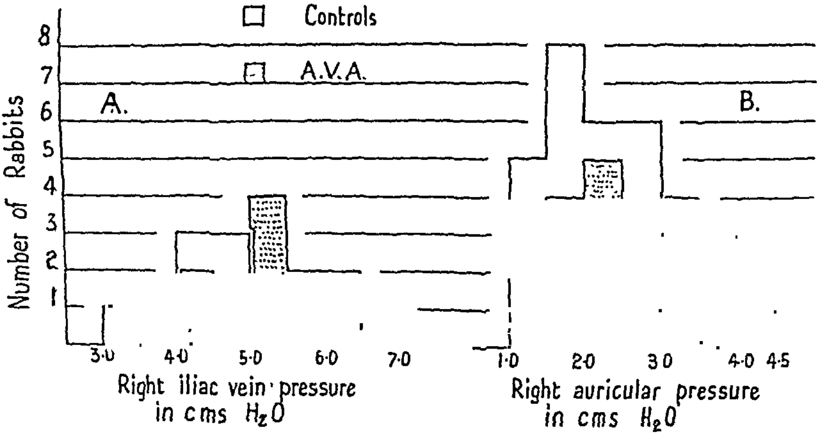

Fig. 5.—Distribution of (A) right iliac vein and (B) right auricular pressures in (1) control rabbits, and (2) those in which the anastomosis had been patent for three months. The level of the great veins in the thorax has been taken as 0 cm. pressure.

manometer to cannulæ inserted into different veins and into the right auricular appendage. The pressure in the veins leading to the superior vena cava, such as the external jugular on the unoperated side, was always raised above normal; the level depending upon the position at which the pressure was taken relative to the leak.

The pressure in the veins leading to the inferior vena cava was measured in the right iliac vein, which was approached through a small abdominal incision. As the animal is tied out on its back this pressure is determined in part by the weight of the intestines; but the values plotted in fig. 5 show that the control series have lower values than those with anastomoses, but that the values of the two series overlap considerably. Moreover, the highest values were not associated with the greatest leaks or with the largest heart weight to carcase weight ratios.

The pressures in the right auricle were measured in the artificially respired animal. The heart was exposed by splitting the sternum and the cannula tied into the tip of the right auricular appendix. This procedure can be carried out without loss of blood or disturbance of the heart beat.

discrete, being arranged at the periphery and leaving a central clear area of sarcoplasm. The interstitial tissue showed no change from the normal.

In the ventricles an increase in fibre diameter could only be appreciated in those hearts in which the hypertrophy was considerable. The interstitial tissue appeared perfectly normal.

Relation of Size of Leak to Degree of Hypertrophy.

Method.

After the animal had been killed, a large cannula, pointing distally, was tied into the aorta close to the heart. This was connected to a bottle containing water at a pressure of 100 mm. of Hg. All arteries were ligated except the carotid leading to the anastomosis, and the inferior vena cava was cut across at its junction with the right auricle. Water was then allowed to flow from the bottle through the anastomosis and out from the cut inferior vena cava, and the amount flowing in a given time noted. Though this gives no idea of the amount of blood passing through the leak during life, it enables a comparative estimate of the size of the leaks to be made.

Results.

In fig. 4 the relationship between the size of the leak and the heart weight to carcase weight ratio is given. It appears from this figure

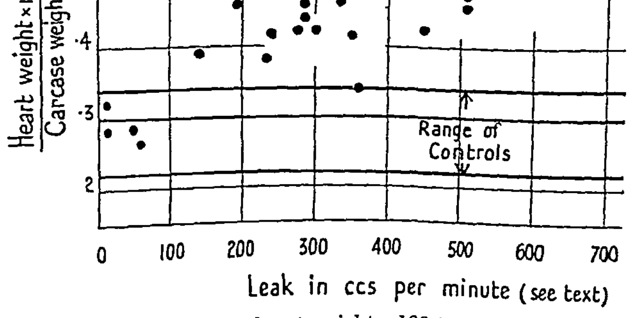

Fig. 4.—Relation of size of leak to heart weight × 100 to carcase ratio in rabbits in which the anastomosis had been patent for three months.

that leaks up to 100 c.c. per mm. have no effect upon the heart, and in consequence all animals having leaks below 100 c.c. have been omitted from the preceding figures. Above this, as the size of the leak increases the hypertrophy tends to become greater, but the relationship

This phenomenon was noted by Lewis and Drury [1923] in a clinical case of arterio-venous aneurysm. It has been seen in every animal in which the closure of the anastomosis has led to a fall in auricular pressure; the rise in peripheral venous pressure therefore occurs with a fall in auricular pressure. The mechanism underlying this reaction has not been fully worked out, but it seems probable that it is due to a redistribution of blood to the lower part of the animal owing to the closure of the anastomosis.

Arterial Pressure.

Using the method described by Grant and Rothschild [1934], the systolic blood-pressure has been measured in the central artery of the ear. It has been consistently observed that immediately after operation the systolic blood-pressure is lower than normal but that it quickly returns to the preoperative level, and is there maintained.

At the final operation, the mean arterial pressure in the right iliac artery was obtained with a mercury manometer, and using a Wiggers capsule the systolic and diastolic pressures were measured in the same vessel (see Table). The mean pressure varied considerably from animal to animal, and upon closing the anastomosis always rose, the rise amounting to about 15–30 mm. Hg. The systolic and diastolic pressures also showed considerable variation from animal to animal. Upon closing the anastomosis there was either a slight or negligible rise in systolic pressure and a definite rise in diastolic pressure, the pulse pressure being always reduced. The difference between the pulse pressure with the anastomosis closed and open is about 10–15 mm. Hg. Into this change the rate of the heart does not enter, for in the animal anæsthetised with urethane no change in heart rate occurs when the anastomosis is closed.

Discussion.

It is evident from these observations that a high degree of auricular hypertrophy and a considerable degree of ventricular hypertrophy is produced in the rabbit's heart when the carotid artery is anastomosed into the jugular vein. The most likely cause for this hypertrophy is a greater cardiac output owing to the increased input due to the raised auricular pressure consequent upon the leak. Actual measurements of the auricular pressure in animals with such anastomoses provide many instances, however, of normal auricular pressures though, through and through, these pressures are raised. It is true that these measurements have been taken on one occasion only, with the chest open and under artificial respiration. The animals, however, judged by the arterial pressures, were in good condition, and the measurements from the iliac

The auricular pressures in the two series which are tabulated in fig. 5 have similar characteristics to those obtained from the iliac vein. In general the values obtained from animals with leaks are higher than in the control series, but values outside the limits observed in the control series are infrequently seen. No relationship could be found between the size of the leak and the auricular pressure. On the other hand, the levels of the auricular pressures and of the iliac veins agreed closely, higher values in the former being found with higher values in the latter.

If the anastomosis is closed the pressure in the right auricle falls abruptly. This fall rarely amounts to more than 1·0 cm. of water, and is usually about 0·5–0·7 cm. of water. It is roughly related to the size of the leak, the greatest falls being observed with the largest leaks (see Table). It may be considered as representing the potential rise in auricular pressure which is imposed by the leak. The actual level of the auricular pressure may be determined not only by this potential rise but also by the ability of the heart to deal with and pass on the greater input.

Heart weight × 100 to carcase weight ratio.	Mean arterial pressure.			Pulse pressure (mm. Hg).			Right auricular pressure (cm. MO).			Leak c.c. per minute.
	A.V.A.		Difference.	A.V.A.		Difference.	A.V.A.		Difference.	
	Open.	Closed.		Open.	Closed.		Open.	Closed.		
·29	112	112	0	50	50	0	2·5	2·5	0	11
·28	90	98	8	39	33	6	2·5	2·3	0·2	50
·42	95	108	13	46	36	10	3·5	3·0	0·5	240
·43	70	84	14	40	30	10	3·0	2·0	1·0	310
·59	105	135	30	107	82	25	3·3	2·2	1·1	380
·43	65	78	13	53	43	10	2·3	1·6	0·7	440
·55	80	105	25	91	76	15	3·9	2·9	1·0	476
·56	76	106	30	76	65	11	2·5	1·3	1·2	616

The pressure in the veins leading to the superior vena cava falls quickly and steadily if the anastomosis is shut off, and returns equally abruptly to its original value if the anastomosis is reopened.

On the other hand, the pressure in the right iliac vein immediately rises when the anastomosis is shut off. The rise usually amounts to about 0·5 cm. of water, and has been found to persist for as long as 10 minutes. During this time, however, in some animals it tends to fall to its original value, but upon opening the anastomosis an additional fall, equal to the rise seen on closing the leak, occurs; the pressure then gradually returns to its original value.

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vein, which were made in the intact animal at the same time, support in general the findings in the right auricle. In all animals the right auricular pressure fell when the anastomosis was closed if the leak was sufficiently large. This indicates that a rise in auricular pressure is occasioned by the leak. It is a more pertinent measurement than that of the actual level of the auricular pressure, for this may well be determined, not only by the potential rise due to the leak but also to the capacity of the heart to handle and pass on the increased input. A rough relation only existed between this potential rise and the degree of hypertrophy, but this may be ascribed to different biological reactions of the hearts to the increased output, for no attempt has as yet been made to limit this variability by the use of litter mates from the same breed or stock. No actual evidence has been presented that the output of the heart is increased, but it has been reported in dogs [Holman, 1937] with moderately large fistulæ, and it may be considered to be present in the rabbits herein reported. The present observations give no information whether the other reason suggested as a cause of enlargement, namely, impaired nutrition due to the low mean or diastolic blood-pressure [Lewis and Drury, 1923], has played a part. In all the animals a simultaneous rise of auricular pressure and fall of mean or diastolic pressure has occurred upon opening the anastomosis. It has been impossible to dissociate the relative importance of these two changes.

If it is true that the increased output of the heart is the main reason for the hypertrophy observed, then the experiments indicate that to maintain this increased output the right ventricle has to hypertrophy a little, but only a little, more than the left ventricle. The auricles, however, show a considerable degree of hypertrophy.

It is clear that studies of the peripheral venous pressures give little or no information, for many animals in which closure of the anastomosis produces a definite change in auricular pressure, normal values have been found in the iliac vein. Moreover, if the anastomosis is closed the peripheral venous pressure rises when it is known that the auricular pressure has fallen.

Finally, very different degrees of hypertrophy have been encountered, and it has not been possible as yet to relate the degree of hypertrophy with any one of the different factors examined.

Summary.

The anastomosis of the right carotid artery into the external jugular vein leads within a short time to cardiac hypertrophy and dilatation.

In the auricles the hypertrophy is considerable, the weights being on occasions four times that of the normal auricle. Both auricles share equally in the hypertrophy.

In the ventricles the hypertrophy is not so great, the weights seldom

being greater than twice the normal. The right ventricle shows a slightly greater hypertrophy than the left.

The change of pressure in the right auricle upon closing the anastomosis indicates that the input into the heart is increased.

When the anastomosis is closed, the venous pressure in the veins leading to the superior vena cava falls, but rises in those leading to the inferior vena cava.

I wish to express my thanks to Dr. T. D. Day and Miss G. Plaut for their help in the histological study of the hearts.

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OBSERVATIONS RELATING TO CARDIAC HYPERTROPHY IN THE RABBIT: THE REFRACTORY PERIOD OF HYPERTROPHIED VENTRICULAR MUSCLE. By ALAN N. DRURY.[1] From the Department of Pathology, University of Cambridge.

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IN a previous paper [Drury, 1936] observations have been reported relating to the "effective refractory period," "full recovery time," and "premature response interval" of ventricular muscle when responding to a series of rhythmic stimuli. It was indicated in that paper that the "effective refractory period" (that is, the earliest moment at which an impulse can be liberated after its predecessor which will be conducted through the muscle) may not necessarily have the same value as the absolute refractory period, for conditions were cited in which there was evidence that very early responses of the muscle may fail to be transmitted. From the point of view of the beat of the heart, which depends upon the spread of the response, the "effective refractory period" as a measurement has greater reality. This measurement can be used comparatively, for no account is taken of the rate at which the earliest responses travel, so that the known errors which vitiate comparative measurements of conduction rates do not enter in. There is a practical difficulty in measuring this period. Immediately after the "effective refractory period" has terminated the excitability of the muscle is considerably depressed, and it is essential that the testing shocks used should be strong enough to excite the depressed muscle. Although the strength of the testing shocks can be used well above the threshold value, it is impossible to be certain that every testing shock has the same value owing to short circuiting with blood, etc., and variations in the measurements may be introduced by this means.

For this reason it was stressed [Drury, 1936] that the "full recovery time" was a much safer measurement to use. The responses to the earliest effective shocks travel slowly to the recording contacts, but as the shocks become less premature this delay decreases till it reaches a constant value. The time-interval between the rhythmic shock and the testing shock at which this constant value first occurs is called

[1] Working on behalf of the Medical Research Council.

the "full recovery time." It is a measurement which is made at a time when excitability has returned almost, if not completely, to normal, and the error due to the strength of the testing shock is thus avoided. It can be used as a comparative measurement, as no account is taken of the actual rate of conduction. Such measurements should therefore be more constant from animal to animal than the "effective refractory period," and the results published in that paper showed this to be the case.

In addition, this delay or "lag" of the early responses, which progressively decreases as the testing shocks become less premature, is such that the interval between the rhythmic responses and the premature responses has a relatively constant value, "premature response interval." This is true until the interval between the rhythmic shock and the testing shock becomes greater than the "full recovery time," when the interval between the responses steadily lengthens. This "premature response interval" has a value very similar to that of the "full recovery time" and provides a very useful check upon the latter measurement. It was finally demonstrated that the "full recovery time" and the "premature response interval" were related to the "effective refractory period." They changed in the same direction as the "effective refractory period" when that period was altered by rate of beating or drugs.

An arbitrary formula was given which allowed the measurements at different rates of rhythmic beating to be compared, the result being expressed as the "final value." This is necessary, because the duration of the "effective refractory period" depends upon the rate of rhythmic stimulation.

$$\text{Final value} = \frac{\text{Full recovery time}}{0{\cdot}10 + \dfrac{\text{inter-rhythmic interval} - 0{\cdot}11}{5}}.$$

In a series of normal animals the final value was close to unity, whereas if the heart was poisoned by drugs the value definitely changed. From the observations reported in that paper it would appear that the three measurements, the "effective refractory period," the "full recovery time," and the "premature response interval," taken in conjunction allow a very safe estimate to be made of the state of ventricular muscle as far as the "effective refractory period" is concerned. If the "final value" of any series of rabbits is close to unity, it may be safely concluded that the ventricular muscle is normal as far as the "effective refractory period" is concerned.

Methods.

The rabbits were anæsthetised with urethane or nembutal and ether if necessary. Under artificial respiration the sternum was split

and the heart exposed. The pericardium overlying the central portion of the right ventricle was cut away and the fish-hook electrodes embedded in the musculature of this region. In other respects the methods used to obtain the required measurements were similar to those already described [Drury, 1936], a modified Ld. II. being used. The rectal temperature of the animal was taken on all occasions and varied between 36·5°–37·5° C. The animal was then killed and the heart fixed and weighed [Drury and Wightman, 1939].

The hypertrophy of the heart was produced by anastomosing the right carotid artery to the right external jugular vein. The animals were kept for three months. The result of this procedure upon the weight of the heart has been fully described [Drury and Wightman, 1939].

Measurements of the "effective refractory period" were made in four different series of animals. The first series was composed of animals in which the anastomosis was never established, or was closed very soon after operation and served as controls. In the second, the leak had been patent for three months, and the hearts were dilated and hypertrophied. In the third, the arterio-venous anastomosis was closed after being patent for three months, and sufficient time was allowed for the dilatation, but not the hypertrophy, to disappear. In the fourth series a similar procedure was adopted, but sufficient time allowed for both the dilatation and the hypertrophy to disappear.

Results.

1. *Control Hearts.*—Although the measurements from a small series of normal rabbits have been already reported [Drury, 1936], they have been repeated as these animals had been subjected to operative procedure and a slight alteration made in the method of stimulation. In Table I. A, the measurements from 10 such rabbits are shown. The average of the final value of this series is 1·04 with extremes of 0·09–0·14. In general the measurements do not differ from those already reported [Drury, 1936, Table VII.]. In the same table the ratios of the ventricular weight × 100 to the carcase weight of the animal, and of the left ventricular weight to the right ventricular weight, are given. In an extensive investigation of rabbits in which the anastomosis quickly closed, it was found that the ratio of the ventricular weight × 100 to the carcase weight varied from 0·18 to 0·32, while that of the left ventricular to right ventricular weight was from 1·9 to 2·8 [Drury and Wightman, 1939]. The hearts tested therefore include examples of high and low ventricular to carcase weight ratios and of high and low L.V./R.V. ratios.

2. *Hypertrophied and Dilated Hearts.*—In Table I. B, the measurements obtained from hypertrophied and dilated hearts brought about by

Table I.—Effective Refractory Period, Full Recovery Time, and Premature Response Interval of Right Ventricular Muscle.

No.	Inter-rhythmic interval in secs.	Effective refractory period in secs.	Full recovery time in secs.	Premature response interval in secs.	Final value.	Ratio of ventricular weight × 100 to carcase weight.	Ratio of left ventricular to right ventricular weight.
			A. Control Hearts.				
0219	0·18	0·083	0·123	0·123	1·08	0·235	2·8
0220	0·15	0·090	0·117	0·122	1·08	0·215	2·0
0222	0·14	0·075	0·111	0·100	0·95	0·290	1·7
0223	0·14	0·080	0·109	0·105	1·03	0·305	2·4
0224	0·20	0·091	0·130	0·137	1·14	0·245	2·3
0217	0·15	0·086	0·111	0·115	1·03	0·306	2·7
0216	0·15	0·073	0·105	0·108	0·09	0·228	2·7
0211	0·15	0·073	0·105	0·107	1·02	0·202	2·5
0363	0·15	0·079	0·110	0·118	1·08	0·260	2·7
0382	0·13	0·087	0·111	0·108	1·07	0·211	2·4
Average	0·15	0·082	0·112	0·114	1·04	0·250	2·4
	0·15	0·089	0·116	0·113	1·04 (From Table VII. [Drury, 1936].)		
			B. Hypertrophied and Dilated Hearts.				
0183	0·15	0·065	0·111	0·108	1·03	0·340	2·1
0372	0·18	0·092	0·120	0·122	1·05	0·505	2·1
0385	0·20	0·097	0·140	0·136	1·16	0·360	2·5
0386	0·15	0·093	0·115	0·120	1·06	0·500	2·4
0391	0·16	0·080	0·118	0·115	1·07	0·430	1·6
0398	0·17	0·087	0·120	0·120	1·07	0·490	1·9
0511	0·20	0·076	0·121	0·122	1·03	0·390	1·9
Average	0·17	0·084	0·121	0·120	1·05	0·449	2·1
			C. Hypertrophied Hearts.				
0560	0·18	0·996	0·123	0·123	1·07	0·405	2·6
0598	0·17	0·087	0·120	0·122	1·07	0·456	2·4
0478	0·14	0·083	0·112	0·110	1·04	0·350	2·0
5484	0·17	0·092	0·118	0·120	1·05	0·333	2·1
Average	0·17	0·089	0·118	0·119	1·05	0·386	2·3
			D. Hearts which have returned to Normal after having been Hypertrophied and Dilated.				
0482	0·17	0·089	0·120	0·116	1·03	0·313	2·6
0501	0·19	0·094	0·126	0·130	1·07	0·304	2·4
0498	0·16	0·082	0·113	0·115	1·04	0·230	2·6
0430	0·16	0·084	0·123	0·120	1·10	0·276	2·4
0514	0·18	0·087	0·125	0·120	1·09	0·320	2·2
Average	0·17	0·087	0·121	0·120	1·06	0·280	2·4

the anastomosis which had been patent for three months, together with the corresponding data of the various heart ratios, are given. In this series the average of the "final value" is 1·04, with extremes of 1·03 and 1·16. When the actual measurements in this series are compared with the control series at the same rate of rhythmic beating, no difference can be detected in the measurements, when it is appreciated that the error in the method may amount to 0·01 of a second in the various measurements taken. The measurements in the slightly enlarged hearts are similar to those found in hearts double the usual size. In some of the hearts there is evidence that the right ventricle had a greater degree of hypertrophy than the left ventricle. The measurements were made on the right ventricle. It can be safely concluded that cardiac hypertrophy and dilatation is not associated with any changes in the measurements.

3. *Hypertrophied Hearts.*—In the same table (Table I. C) the measurements from four animals are included in which the anastomosis, after being patent for three months, was closed some days before the measurements were made. During this period there was a very definite decrease in the area of the X-ray shadow of the heart, indicating that the dilatation had become much less, if it had not disappeared completely. At the same time the heart weight to carcase weight ratios show that hypertrophy is still present. In these animals also there is no indication of any change in the measurements.

4. *Hearts which have shown Hypertrophy and Dilatation but which have returned to their normal size after removal of the Arterio-venous Anastomosis.*—In this series (Table I. D) the anastomosis, after being patent for three months, was closed some weeks before the measurements were made. The X-ray shadow of the hearts obtained just prior to the closure was of the same order as that seen in the second series, so that a similar degree of hypertrophy and dilatation had been reached at this stage. At the time the measurements were made the X-ray shadow was within normal limits, so that no dilatation was present. The ratios of the ventricular weights to the carcase weights are all within, but on the upper side of, the normal limits.

Discussion.

The evidence put forward in this paper shows that the "effective refractory period" of hypertrophied and dilated ventricular muscle experimentally produced does not differ from that found in normal muscle. The results in human hearts showing dilatation and hypertrophy indicate also that there is no change from the normal in the refractory period. The refractory period and ventricular systole terminates just before the end of the final deflection T of the electrocardiogram, and the Q–T interval gives a measure of the refractory

period [Herman and Wilson, 1926]. As the Q–T interval in cases with considerable enlargement of the heart is within normal limits [White and Mudd, 1929], it may be said that cardiac enlargement in clinical subjects is not associated with an increase in the refractory period.

In the series of rabbits in which arterio-venous anastomoses have been made, cardiac failure with œdema and ascites has been occasionally observed. The human hypertrophied heart is prone to failure, so that there appears to be a similar functional disability in both the human and the experimentally produced hypertrophied hearts. The human hypertrophied heart, moreover, fails quickly if the rate of beating is maintained at a high rate. It has been suggested that the failure is due to the fact that the period of rest between the beats is insufficient for the full recovery of the muscle [Harrison, 1935]. The results reported indicate that the changes necessary for the return of the responsive state take place as quickly in hypertrophied as they do in normal muscle. The disability which leads to failure envisaged in this hypothesis must concern a different set of recovery processes which are not reflected in the duration of the "effective refractory period."

Summary.

The "effective refractory period" of ventricular muscle which is (1) hypertrophied and dilated, (2) hypertrophied, (3) has returned to normal after being hypertrophied and dilated, is within normal limits.

I wish to express my thanks to Mr. J. A. F. Fozzard, of the Department of Anatomy, for carrying out the X-ray work.

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LIBERATION OF ACETYLCHOLINE FROM THE PERFUSED CAT'S BRAIN.

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THE experiments described in this paper were undertaken in order to find whether a liberation of acetylcholine from the living brain could be demonstrated. Much evidence has accumulated indicating that this substance is the chemical transmitter across synapses in sympathetic ganglia, and the possibility that it plays a similar rôle in the central nervous system has been frequently discussed. The various central effects produced by acetylcholine and eserine, the presence of the former substance and of choline esterase in the central nervous system, and the property of this tissue to synthesise acetylcholine, have been quoted in support of this hypothesis. Nachmansohn [1939] has found not only a strong concentration of choline esterase in the grey matter, but also that the enzyme appeared at the stage of development at which the synapses begin to function. Synthesis of acetylcholine has been found to occur in tissue slices and in a suspension of cells and cell debris from brain [Quastl *et al.*, 1936; Mann *et al.*, 1938; Trethewie, 1938].

Experiments to study the liberation of acetylcholine from the central nervous system have given equivocal results. Dikshit [1934] found in cats that the cerebro-spinal fluid collected during central vagus stimulation sometimes contained more acetylcholine than the fluid collected before stimulation. Feldberg and Schriever [1936] were unable to confirm these findings. Negative results were also obtained by Adam *et al.* [1938], who perfused the ventricular system of the dog's brain with eserinised Locke solution and stimulated the vagus, other sensory nerves, different parts of the brain, and the spinal cord. According, however, to Chang *et al.* [1937, 1938 *a*, *b*), acetylcholine does appear during central vagus stimulation in the venous blood from the isolated dog's and cat's head vivi-perfused by a second eserinised animal. In later experiments on dogs, Chang *et al.* [1938 *c*]

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succeeded in demonstrating the appearance of acetylcholine, or its increase, in the cerebro-spinal fluid during central vagus stimulation when the eserine had not only been given intravenously, as in the experiments of Feldberg and Schriever, but also intraspinally. Strong faradic stimulation of the isolated spinal cord of rabbits [Minz, 1936] and of the brain-cord preparation of toads [Li, 1939] was also found to be associated with the appearance in the surrounding bath fluid of a substance resembling acetylcholine. According to Li, a constant current passing through such a preparation had no effect of this kind.

In the present experiments the effect of KCl in liberating acetylcholine from the perfused cat's brain was studied. In addition some observations on the spontaneous liberation of acetylcholine were made. KCl has central excitatory effects in small doses and paralysing effects in larger ones [Bonnet and Bremer, 1937]; it has been shown to liberate acetylcholine from various organs supplied by cholinergic nerves [Beznak, 1934; Brown and Feldberg, 1936; Feldberg and Guimarais, 1936], and even from tissue slices or a suspension of cells or cell debris obtained from the brain of rats and guinea-pigs [Mann *et al.*, 1939]. In so far as concerns the liberation of acetylcholine, intra-arterial injections of KCl have been regarded as an imitation of the arrival of impulses at the endings of cholinergic nerves, but recent observations of Brown and MacIntosh [1939] on the perfused ganglion have shown that injections of KCl cause repeated discharges from nerve fibres in continuity, as well as from nerve cells. They therefore question whether certain effects of injected KCl were rightly attributable to stimulation of the ganglion cells. It is possible that the liberation of acetylcholine is also not only a direct effect of KCl, but results in part secondarily from such nervous discharges. In both cases the potassium salt should provide an appropriate stimulus for studying the liberation of acetylcholine in the brain.

METHOD.

The cat's head was perfused through the carotid arteries with defibrinated blood according to the method described by Chute and Smyth [1939]. It has been shown [MacIntosh, 1939] that the liberation of acetylcholine from the superior cervical ganglion of the cat can be consistently demonstrated when the ganglion is perfused with Locke's solution or diluted blood, but not when perfused with full blood. We have therefore used 150 c.c. blood diluted with an equal volume of saline. Since it was necessary to prevent mucus and saliva from contaminating the blood, the salivary glands were removed as far as possible and the nasopharynx was plugged. There remained some secretion from the mucus glands of the palatine mucosa which was collected on a pad of cotton-wool. This pad was changed from time

to time during the experiment. In control experiments in which the brain was excluded from the circulation the preparation was made in the usual manner, and the head first perfused for some minutes with the brain intact. The arterial supply was then cut off, the brain removed through a small opening in the roof of the skull and the cranium packed with plaster of paris. When the perfusion was restarted after hardening of the plaster (10–15 minutes) there was a venous outflow from the remaining tissue. In some experiments, both with and without the brain, the original method of Chute and Smyth was modified by reducing the amount of extra-cranial tissue perfused. This was done by tying the infraorbital vessels near their origin and by placing mass ligatures about the base of the eyeballs. In order to prevent the destruction of acetylcholine liberated in the brain, eserine to give a concentration of 1/300,000–1/400,000 was added to the blood in the reservoir. The potassium chloride was injected into the arterial cannulæ through the rubber tubing, which was momentarily clamped distal to the point of injection, 20–25 mg. KCl, in 0·5 c.c. distilled water, being injected simultaneously into each side. For the assay of acetylcholine 10–20 c.c. samples of blood were taken; 300 c.c. of blood was available of which 100 c.c. was required to fill the perfusion system. The blood was collected directly from cannulæ in the jugular veins into graduated cylinders containing one drop 1/1000 eserine, and, if not to be tested immediately, kept in a bath of ice water. In carrying out the tests, the blood, in amounts of 1 to 2 c.c., was injected intravenously into eviscerated cats under chloralose anæsthesia, and the effects on the arterial blood-pressure were compared with those produced by known amounts of acetylcholine chlorides. The sensitivity of our cats was usually such as to enable us to detect in the blood concentrations of acetylcholine of 1 in 400 millions and sometimes 1 in 800 millions. The blood in some experiments was also tested on the eserinised leech preparation.

Results.

1. *Eserine.*

When the head was perfused with 50 per cent. blood without eserine, the reflex activity of the eyes disappeared more rapidly than when the head was perfused with full blood. The wink reflex diminished after 30 to 40 minutes and disappeared in an hour. The addition of eserine to the blood caused in the beginning a marked potentiation of existing reflexes or their reappearance if they had disappeared, and under good conditions spontaneous bilateral blinking was noted. In one experiment the rate of blinking was timed and found to be about 80 per minute. These movements were sometimes associated with jerking movements of the head. When the facial nerve was cut on one

side, spontaneous opening and closing of the lids occurred only on the innervated side, indicating a central factor in the production of this effect. After eserine we also observed twitchings of the facial muscles, eye movements and tear secretion. At later stages of eserine poisoning the hyper-excitability diminished, and eventually all spontaneous movements and all reflex activity were abolished. The attainment of this condition was dependent on the concentration of eserine in the blood, appearing earlier with stronger concentrations. Similar effects of eserine have been described in the whole animal by Schweitzer and Wright [1936]. In spinal cats and in cats under chloralose anæsthesia eserine produced increased reflex-excitability and centrally evoked convulsions followed by depression.

Assay of Acetylcholine.—When, before the addition of eserine, the venous effluent was tested on the arterial blood-pressure of the cat, it usually produced not a fall but rather a slight rise. In some early experiments the blood had a depressor action which, unlike that of acetylcholine, was not abolished by atropine. This effect was probably caused by contamination of the blood with mucus or saliva, which is known to contain such a depressor substance. When precautions were taken to guard against such contamination the atropine resistant depressor action was no longer obtained.

Samples of blood taken after the addition of eserine and tested on the cat's blood-pressure usually had a depressor action which was abolished by atropine. When compared with acetylcholine the depressor activity corresponded to that of a concentration in the blood of 1 in 400 million to 1 in 800 millions. Samples taken at 15-minute intervals showed almost no increase in depressor activity, but a slight gradual increase was demonstrable if longer intervals were employed, and was particularly noticeable if the volume of the perfusing blood was kept as small as possible.

The blood also had a stimulating action on the eserinised leech muscle, and when compared with acetylcholine the activity was of the same order as that obtained by the assay on the cat's blood-pressure. The substance is, therefore, probably acetylcholine, and must have been liberated from the brain, since in the two control experiments in which the head was perfused without the brain no depressor substance appeared in the eserinised blood even after an hour's perfusion (fig. 2, D).

2. *Potassium.*

The arterial injections of KCl caused midriasis and pronounced diminution of venous outflow, both effects passing off within a few minutes. The injections were followed by a temporary disappearance of the reflex activities of the eye.

Assay of Acetylcholine.—Immediately prior to the injection of

potassium a venous sample was taken and used as a control. When the outflow began to decrease a few seconds after the injection a first sample of about 20 c.c. was collected. The flow had nearly always returned to normal before the collection was completed. Even if all the injected potassium had been recovered in this sample the amount in 2 c.c. would only be 5 mg., which we found did not interfere with our assay on the cat's blood-pressure. A second venous sample was taken immediately following the first, and subsequent samples were taken at intervals of 5 to 15 minutes.

The venous samples collected after the injection of the KCl always showed a greater depressor action than the controls. The effect was

Fig. 1.—Cat under chloralose. Carotid blood-pressure. Stomach, intestine, and spleen removed. At A to E and at G to K, 2 c.c. of venous blood from perfused cat's head. At F, 2 c.c. acetylcholine 1 in 400 millions. Between J and K, 1 mg. atropine intravenously. For details see text.

most pronounced in the first, second, or third sample following the injection. The depressor activity of later ones diminished, but remained greater than that of the control. When arterial blood samples were removed from the reservoir during the first 10 to 15 minutes after the injection, they were found to be less active in causing a fall in blood-pressure than the venous samples. At later stages the difference between arterial and venous blood disappeared, both giving a depressor effect greater than that of the control. A second injection of potassium chloride produced similar effects, causing a still further increase in depressor activity. The depressor action of all samples was abolished by atropine, and was therefore probably due to acetylcholine. A typical experiment is illustrated in fig. 1, in which are seen the depressor effects of successive 2 c.c. samples collected from the veins of a cat's head perfused with 50 per cent. blood containing eserine in a concentration of 1 in 300,000. The blood collected before the injection of KCl had a slight depressor effect (at A); after the injection it became progressively stronger in the first two samples collected (at B and C), decreasing again in the subsequent two samples (at D and E). Perfusion was continued for some time before the sample tested at G was collected. The depressor activity of the blood had not materially changed within

side, spontaneous opening and closing of the lids occurred only on the innervated side, indicating a central factor in the production of this effect. After eserine we also observed twitchings of the facial muscles, eye movements and tear secretion. At later stages of eserine poisoning the hyper-excitability diminished, and eventually all spontaneous movements and all reflex activity were abolished. The attainment of this condition was dependent on the concentration of eserine in the blood, appearing earlier with stronger concentrations. Similar effects of eserine have been described in the whole animal by Schweitzer and Wright [1936]. In spinal cats and in cats under chloralose anæsthesia eserine produced increased reflex-excitability and centrally evoked convulsions followed by depression.

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solutions or very dilute blood on account of the early onset of cerebral œdema. In our experiments where a compromise was made by using blood diluted with an equal volume of saline, the small but definite amount of acetylcholine demonstrated is significant. No attempt has been made to determine from which part of the brain it was liberated, nor could the liberation be correlated with special nervous activity. The doses of potassium used for demonstrating the liberation of acetylcholine were, in fact, paralysing ones. This might at first sight appear contradictory to the conception that liberation of acetylcholine is associated with activity of the central nervous system. The difficulty, however, can easily be explained. We know from the experiments on the perfused cervical ganglion that paralysis by KCl affects the nerve cells without impairing the mechanism of liberating acetylcholine at the synapses, at least not in the early stages of paralysis. The paralysis is comparable to the well-known forms of paralysis caused by atropine, nicotine, or curare, where the effector cells cease to respond to nerve stimulation despite the fact that the liberation of acetylcholine remains unaffected.

Summary.

The almost completely isolated cat's brain was perfused with 50 per cent. defibrinated blood. The addition of eserine to it produced increased reflex-excitability with spontaneous movements followed by depression, and caused the appearance in the blood of small amounts of acetylcholine which were liberated from the brain. This liberation was temporarily increased by the injection of KCl.

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this time. It was still stronger than that caused by the blood collected before the injection. A new injection of KCl was given, and two subsequent samples were collected and tested at H and J. The depressor activity was abolished by atropine, as seen at K, where the sample H was retested. In the control experiments in which the brain was removed KCl failed to cause the appearance of any depressor substance in the venous blood. In the experiment from which fig. 2 is taken the head was perfused without the brain and after ligating the infra-orbital vessels and the eyeballs. The perfusion fluid was again 50 per cent. blood containing eserine in a concentration of 1 in 300,000. The control blood (at A) and that collected in three successive samples

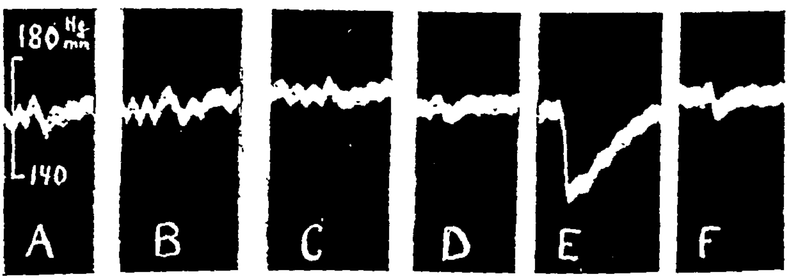

Fig. 2.—Cat under chloralose. Carotid blood-pressure. Stomach, intestine, and spleen removed. At A to D, 2 c.c. of venous blood from perfused cat's head without brain. At E, 2 c.c. acetylcholine 1 in 400 millions. At F, 2 c.c. of saline. For details see text.

after the injection of KCl (at B, C, and D) contained no detectable amounts of acetylcholine. The sensitivity of the cat's blood-pressure was such that a concentration of acetylcholine of 1 in 400 millions (at E) would have been detected. The slight fall of 2–3 mm. obtained with all samples is an unspecific effect which was also obtained when saline was injected (at F).

These experiments suggest that the slight spontaneous liberation of acetylcholine from the brain perfused with eserinised blood is greatly increased by the injection of KCl, and that the augmented output continues for some minutes, raising the acetylcholine concentration of the blood in the reservoir.

Discussion.

One of the difficulties in demonstrating the liberation of acetylcholine from the brain during activity consists in the fact that conditions which favour its detection are incompatible with the normal activity of the central nervous system. Even in the ganglion it has been found necessary to dilute the perfusing blood in order to obtain consistent results. It is not possible to perfuse the brain with salt

solutions or very dilute blood on account of the early onset of cerebral œdema. In our experiments where a compromise was made by using blood diluted with an equal volume of saline, the small but definite amount of acetylcholine demonstrated is significant. No attempt has been made to determine from which part of the brain it was liberated, nor could the liberation be correlated with special nervous activity. The doses of potassium used for demonstrating the liberation of acetylcholine were, in fact, paralysing ones. This might at first sight appear contradictory to the conception that liberation of acetylcholine is associated with activity of the central nervous system. The difficulty, however, can easily be explained. We know from the experiments on the perfused cervical ganglion that paralysis by KCl affects the nerve cells without impairing the mechanism of liberating acetylcholine at the synapses, at least not in the early stages of paralysis. The paralysis is comparable to the well-known forms of paralysis caused by atropine, nicotine, or curare, where the effector cells cease to respond to nerve stimulation despite the fact that the liberation of acetylcholine remains unaffected.

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THE INFLUENCE OF NERVES AND DRUGS ON SECRETION BY THE SMALL INTESTINE AND AN INVESTIGATION OF THE ENZYMES IN INTESTINAL JUICE.

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PART I. THE INFLUENCE OF NERVES AND DRUGS ON SECRETION.

IT is generally agreed that direct mechanical and chemical stimulation of the intestinal mucosa causes a secretion of *succus entericus*. There is also some evidence that hormonal influence is involved, but the rôle of the vagus and sympathetic nerves is not clear.

In the following paper experiments will be described dealing with the influence of extrinsic nerves and hormones on the production of succus entericus.

EXPERIMENTAL.

Extrinsic Nerves. (1) *The Vagus.*

Savitch and his co-workers [1917, and cited by Babkin, 1928] have reported experiments on the influence on intestinal secretion of stimulation of the vagus nerves in the neck of decapitated cats. The animals were placed in a saline bath at 37° C. and the small intestine was milked downward with the fingers at regular intervals. Under these conditions the unstimulated control animals began to produce fluid after 4–5 hours. After stimulating the vagus nerve in the neck the secretion appeared after the long latent period of 1–1½ hours, and, though stated to be dependent on the continuation of stimulation, the experimental records reproduced by Babkin do not indicate very clearly that this was so. These would appear to be the only experiments reporting positive effects from vagal stimulation.

In the following experiments decerebrate or decapitate cats have been used exclusively, for, as will be seen later, anæsthetics have a profoundly depressing action on intestinal secretory phenomena. The animals were starved for 24 hours before operation. Under ether anæsthesia, with artificial respiration, the chest was opened along the eighth right costal interspace, and the eighth rib divided near the

vertebræ. The vagus nerves were dissected below the lung roots where they lie beside the œsophagus. They were tied, cut and the peripheral ends drawn on to protected platinum electrodes. Fine enamelled wires from these electrodes were passed through the chest wall, the lungs were fully inflated and the chest closed. Through a midline abdominal incision, the accessory pancreatic duct, the main pancreatic duct and the common bile duct were tied. When the duodenum only was under investigation the pylorus was closed either by a glass ball or by a ligature embracing the mucosa applied through a small longitudinal incision in the muscle just proximal to the pyloric sphincter. Cannulæ were tied into the stomach and into the duodenum 6 centimetres from the pylorus. When the jejunum or ileum was being investigated as well as the duodenum, a glass obturator in the form of a cone was passed into the duodenum and secured to its wall 6 centimetres from the pylorus, thus isolating the duodenum from the rest of the small intestine without interrupting the continuity of the intestinal wall. The cone was placed in position by attaching it to a thread which passed through the eye in the tip of a probe; the end of the probe was inserted through a hole in the stomach wall and guided through the pylorus and down the intestine for 6 centimetres. A small cut was made over the tip of the probe and the thread drawn out from the eye. The obturator could then be drawn into position in the gut lumen by means of the thread and fastened by a small stitch to the intestinal wall. The duodenum was drained in a retrograde direction by introducing the end of a straight cannula with a bulb in the middle through the pylorus in a similar way. The introducing thread was passed through the belly wall and later was attached to a weight so that the bulb of the cannula was held tightly against the pylorus to isolate the duodenum from the stomach. A rubber tube attached to the proximal end of the cannula passed through the hole in the stomach through which the obturator and cannula had been introduced. A cannula was tied into the stomach and another into the small intestine at the required distance below the obturator. The intestines were returned to the abdominal cavity and the rubber tubes attached to the cannulæ were brought through appropriately placed stab wounds in the abdominal wall. The abdominal wound was closed or its edges were sewn to a perspex window [Wright *et al.*, 1938]. By this technique two different parts of the intestine were cannulated without seriously interrupting any of the nervous pathways. At the end of the experiment the competence of the obturators was tested by introducing pontamine blue into the intestine above them.

After preparing the intestines the cat was decerebrated and left for 2 hours to allow the effects of the anæsthetic to wear off, the temperature being maintained at 37°–38° C. Stimulation was then begun at the rate of 30 faradic impulses per second for periods of

14 and 11 seconds, separated by intervals of 15 and 20 seconds and at such a strength that a definite shock was given to the tongue.

Within a few minutes gastric juice began to flow from the gastric, and a sticky juice from the duodenal, cannula. The highest rate of flow from the duodenum was 8·4 c.c. per hour and in that hour the stomach secreted 26 c.c. of juice. The smallest rate of flow from the duodenum was 2 c.c. per hour associated with a gastric flow of 20 c.c. per hour. The flow continued as long as the stimulation, ceased when it ceased and began again on its recommencement. In all, 28 experiments were performed and in none was there a failure to elicit secretion of juice following stimulation. In none of the experiments was there a flow of juice from the jejunum though it had been kept in anatomical continuity with the duodenum. The detailed results of two such experiments were as follows:—

Cat 62. Weight 3·2 kg.

10.45. Ether anæsthesia. Tracheal cannula tied in and threads looped round carotids. Chest opened on right side. Infracardiac vagi secured, dissected and placed on electrodes which were left in the chest with leads through the wall.

Abdomen opened by upper midline incision. Common bile duct and pancreatic duct tied. Stomach opened, pyloric obturator introduced, stomach sewn up and sutured to abdominal wall to give counterpoise to the obturator. Duodenum cut across 6 cm. from pylorus and cannula introduced and tied in. Window sewn in.

Carotids tied and decerebration completed at 12.15.

14.30. Cannula dry, duodenum inactive. Stimulus commenced—immediately contractile activity became apparent in the duodenum (stomach not visible) with dilatation of vessels and in 5 minutes bile-stained fluid, sticky and alkaline, was coming from the cannula. The fluid soon became colourless with slight opalescence.

15.20. 4·5 c.c. collected.
16.30. 6·0 c.c. ,,
17.30. 6·0 c.c. collected. Stimulation stopped.
19.00. No further secretion. Stimulation recommenced.
22.00. 12 c.c. more collected. Cat dead.
50 c.c. strongly acid fluid in stomach.

Cat 71.

11.00. Ether anæsthesia. Tracheal cannula inserted. Thorax opened with artificial respiration. Infracardiac vagi secured and placed on electrodes. Common bile duct, pancreatic duct and accessory pancreatic duct tied. Submucous tie on pyloric mucosa. Duodenum and stomach cannulated. Window sewn in and decerebration completed 12.15.

14.45. No secretion from stomach or duodenum.

15.00. Stimulation started. Good motor activity of stomach and duodenum.

	Duodenum.	*Stomach.*
16.00.	4·4 c.c. slightly bile-stained sticky fluid. 1 c.c. neutralises 0·2 c.c. N/10 HCl.	20 c.c. clear mucoid gastric juice.
17.00.	5·3 c.c. clean clear juice.	22 c.c. ,, ,,
18.00.	6·0 c.c. ,, ,,	25 c.c. ,, ,,

Stimulation stopped. 40 c.c. saline given *subcutem.*

19.00. No secretion: stimulation recommenced.

20.30. Secretion had collected but was spilled by movement of animal.

21.30. 3·0 c.c. duodenal juice. Stimulation stopped.
0·6 mg. eserine *subcutem.*

21.50. 0·6 mg. eserine *subcutem.*

22.00. Stimulation recommenced.

23.00. 5·5 c.c. clear fluid.
Cat died at 23.10.

In three experiments a cannula was tied into the jejunum instead of the duodenum. There was no flow of juice on stimulation. After stimulation for 2 hours the duodenum was severed 6 centimetres from the pylorus and cannulated; further stimulation then gave a flow of juice from the duodenum. It was thus apparent that the secretion had been forming in the duodenum and was being absorbed in the jejunum. Another experiment also illustrated that complete re-absorption of previously secreted juice is possible. Duodenal juice (6·0 c.c.), produced by vagal stimulation, was returned to a jejunal loop after atropinisation of the animal. It was completely absorbed in 2 hours 20 minutes; possibly the absorption was quicker than this but the preparation was not examined sooner.

It was thought that the whole of the secretion might be coming from the Brunner's glands of the duodenum, so a glass cone was fixed in the duodenum 2 centimetres only from the pylorus and both ends of the duodenum cannulated in the appropriate way. Stimulation of the vagi gave a good flow of juice from that part containing Brunner's glands and a lesser but still definite secretion from the duodenum containing none. The details of one of three experiments illustrating these points were as follows:—

Cat 100. Weight 2·5 kg.

10.30. Ether anæsthesia. Electrodes applied to infracardiac vagi. Common bile duct, pancreatic duct and accessory pancreatic duct tied. Obturators and cannulæ inserted. Decerebration completed at 11.45.

14.30. Animal in fair condition. Stimulus started.

17.30. Stomach—30 c.c. of clear mucoid secretion, 1 c.c. equivalent to 1·5 c.c. N/10 HCl. Upper duodenum—6 c.c. of clear slimy alkaline secretion, 1 c.c. neutralises 0·2 c.c. N/10 HCl; no precipitation with N/10 HCl. Lower duodenum—2·4 c.c. of slightly turbid secretion, almost watery in consistence and just alkaline: no neutralising value; turbidity on adding N/10 HCl.

Path of Vagus Supply to the Duodenum.—The path of the vagus supply to the duodenum was investigated by setting up an animal as above with the exception that two strong ligatures were left untied around the pylorus. After the rate of secretion on stimulation had been determined over 2 hours the ligatures were tied tightly and the pylorus cut through between them, care being taken to avoid interference with the blood supply. Further stimulation gave no significant secretion from the duodenum. Cutting the mucosa and submucosa only did not interfere with secretion. It was thus clear that the vagal supply to the upper duodenum passed from the stomach in the muscular layer of the gut wall.

Effect of Eserine on Vagal Stimulation.—The rate of secretion of duodenal juice during vagal stimulation was increased markedly by two doses of eserine (0·6 mg. each, given at an interval of 20 minutes), but it will be noted later that eserine in these doses may excite of itself a secretion of juice from the duodenum, jejunum and ileum.

In view of the failure to obtain juice from the jejunum or ileum by vagal stimulation attempts were made to "sensitise" the small intestine to the influence of the vagus. Eserine (0·6 mg.) was given *subcutem* to a cat and then 2 hours were allowed to elapse to see whether the eserine by itself produced a secretion. If there was no secretion the vagi were then stimulated, but no positive effects were obtained.

Action of Atropine.—Atropine (1·0 c.c. of 1 p.c. atropine sulphate) immediately caused a cessation of vagal secretion which lasted, even though stimulation was continued, for at least three hours.

The Effects of Anæsthetics.—The effect of basal anæsthetics on the rate of secretion from the duodenum was investigated in decapitated cats. After the rate of secretion had been determined by a two-hour control period of stimulation, 150 mg. of sodium luminal per kilo of body weight was given intramuscularly. Though this was not sufficient to cause a disappearance of the anal reflex, it caused almost complete cessation of secretion. This lasted during 3–4 hours of vagal stimulation. Administration of eserine gave some return of the vagal secretory effect. Details of such an experiment are as follows:—

Cat 169. 4·3 kg.

11.00. Ether anæsthesia. Decapitation. Vagi placed on electrodes. Common bile duct, pancreatic duct and accessory pancreatic

duct tied. Obturator placed in pylorus. Stomach and duodenum cannulated. Completed at 12 noon.

15.00. Stimulation started.
16.00. Collection started.
17.00. Stomach 17·5 c.c., 1 c.c. equivalent to 1·5 c.c. N/10 HCl.
Duodenum 5 c.c.
17.15. 650 mg. sodium luminal intramuscularly.
17.40. Anal reflex still present. Collection started.
18.40. Stomach 4 c.c., 1 c.c. equivalent to 1·5 c.c. N/10 HCl.
Duodenum 0·2 c.c.
Given eserine gr. 1/100 *subcutem.*
19.00. Eserine gr. 1/100 *subcutem.*
20.00. Gastric juice 6 c.c.
Duodenal juice 1·5 c.c.

Nembutal gave similar effects as follows:—

Cat 171. 2·8 kg.

9.45. Ether anæsthesia. Vagi dissected and placed on electrodes. Accessory pancreatic duct, common bile duct and pancreatic duct tied. Obturator placed in pylorus. Stomach and duodenum cannulated. Decerebration 10.55.

12.25. Stimulation of vagi started.
13.05. Secretion from both cannulæ flowing freely.
Collection begun.
14.05. Stomach 18 c.c.
Duodenum 3·6 c.c.
Cat given 1 c.c. nembutal (0·07 gm.) intravenously.
14.30. Collection started.
15.30. Stomach 4 c.c.
Duodenum 0·3 c.c.
15.40. Eserine gr. 1/100 *subcutem.*
16.00. Eserine gr. 1/100 *subcutem.*
16.20. Collection started.
17.20. Stomach 6 c.c.
Duodenum 1·2 c.c.

Ether similarly acted as an inhibitor. When a decerebrate animal had reached a steady rate of secretion of 20 c.c. per hour from the stomach and 5 c.c. per hour from the duodenum as a result of vagal stimulation, ether was administered for 1 hour. During this time scarcely any secretion occurred (stomach 3 c.c., duodenum 0·3 c.c.) in spite of the continuation of stimulation. The animal was left for 2 hours to allow elimination of the ether and then the vagi were stimulated again. Though by this time the animal's condition was poor it gave 10 c.c. of gastric secretion and 2·5 c.c. of duodenal secretion in an hour.

Of the anæsthetics tested, chloralose had the least effect on duodenal secretion. The rate of secretion in a decapitate cat during vagal stimulation was 8·4 c.c. per hour. After chloralose was given (70 mg. per kilo body weight) the rate of secretion was reduced to 5·5 c.c. per hour.

Some Properties of the Secretion.—The secretion which was obtained from the duodenum by vagal stimulation was a slightly opalescent, slimy fluid; at first it was bile-stained, but after 2 or 3 hours' flow it became colourless or only faintly yellow. It was alkaline to litmus and on collection had a pH of 8·3–8·4. On adding excess HCl and titrating back with NaOH using phenolphthalein as an indicator, 1 c.c. gave a neutralising value equivalent to 0·25–0·3 c.c. N/10 HCl. The chloride content was 0·45 p.c. There was only a slight increase of haziness on addition of N/10 HCl or 2 p.c. acetic acid, but the bulk of the mucus could be precipitated by phosphotungstic though not by trichloracetic acid. On standing on the bench overnight the sliminess disappeared from the juice. Vagal juice, as will be seen later, is indistinguishable from juice collected from a duodenal fistula.

From this series of experiments it is clear that stimulation of the vagi by the method used always causes secretion from the duodenum but not from the rest of the small gut, even when this is "sensitised" by a dose of eserine. The secretion from the duodenum is principally from the portion just distal to the pylorus, in which Brunner's glands are present.

The possibility existed that the secretion of the lower portion of the small intestine was under the control of the nervi erigentes, stimulation of which causes secretion in the colon [Wright *et al.*, 1938]. In one experiment done to investigate this point nothing was obtained from the ileum though good colonic secretion occurred.

(2) *The Sympathetic.*

Claude Bernard [1859] mentioned that the removal of the mesenteric (solar) ganglia caused a production of fluid in the intestine accompanied by diarrhœa. This phenomenon was more fully investigated in the dog by Moreau [1868] who found that section of the nerves accompanying the mesenteric vessels caused the accumulation of fluid in the loop of small intestine so denervated. This experiment has been confirmed by a number of workers who, for the most part, consider that the properties of the fluid entitle it to be considered true *succus entericus* [Hanau, 1886; Mendel, 1896; Falloise, 1904; Molnár, 1909 (using a denervated Thiry fistula)]. Leubuscher and Tecklenburg [1894] and Tecklenburg [1894] also repeated the experiment but concluded that the fluid was a transudate, a view supported by Starling [1911] who,

however, admitted that no proof was available against the view that secretion is inhibited by the sympathetic nerves.

The most thorough investigation of the nervous mechanisms involved was carried out by a committee of the British Association [Pye-Smith *et al.*, 1874; Brunton and Pye-Smith, 1875, 1876]. They also gave a résumé of the earliest work on this subject. They concluded that: "The secretory nerves of the intestines have the small ganglia of the solar and superior mesenteric plexuses for their centres; hence secretion is unaffected by section of the splanchnics, the vagi, or the dorso-lumbar part of the cord."

Mitsuda [1924], as the result of some not very convincing experiments, thought that the extra-intestinal parasympathetic is the secretory nerve for the intestine and that the extra-intestinal sympathetic is principally inhibitory to the glands by means of intra-mural sympathetic ganglia.

In the following section we record experiments dealing with this "inhibitory" influence of the sympathetics on secretion.

Effects of Cutting Greater Splanchnic Nerve.—For these experiments the chest was opened in the tenth interspace and the tenth rib divided near the transverse processes of the vertebrae. One pair of intercostal arteries were doubly ligated near the aorta and cut. Dissection just above the diaphragm then exposed a sufficient length of the splanchnic nerves to allow them to be cut and, if desired, drawn on to protected electrodes. The gut was prepared in the same way as for vagal stimulation. The animal was then decerebrated and left for the effect of the anæsthetic to wear off. It was found that after a variable period of from 1 to 5 hours the duodenum began to expel juice from the cannula. The highest rate of secretion from the duodenum was 6 c.c. per hour. There was no secretion from the jejunum, but occasionally the animal showed evidence of increased intestinal motility by passing fæces from the rectum.

The variable delay appeared to be due to the anæsthetic, for if the intestines of the animal were set up as described and then the cat was decerebrated and left for 2 hours before section of the sympathetic nerves (both can be rapidly divided *via* a short incision in the eleventh right intercostal space), the flow of secretion from the duodenum began at once. In some cases before the thoracic incision could be sewn up fluid was escaping from the duodenal cannula. This possible effect of anæsthetics was commented on by Mendel and Falloise.

This activity of the duodenum was not dependent on the continuity of the vagus nerves with the medulla, for if the vagi were divided below the lung root at the time of the initial operation division some hours later of the splanchnic nerves still gave rise to a rapid onset of secretion from the duodenum. Division of the vagi during "paralytic" secretion

had also no effect. The following protocol illustrates some of these points:—

Cat 144. 3 kg.

11.00. Ether anæsthesia, chest opened, splanchnics divided, chest closed. Common bile duct, pancreatic duct and accessory pancreatic duct tied. Obturator placed in pylorus. Duodenum 6 cm. from pylorus and stomach cannulated. Decerebration 12 noon.

17.00. 6 c.c. of secretion from stomach.
22 c.c. of sticky secretion from duodenum, 5·3 c.c. in the last hour.
Chest reopened and vagi divided without anæsthetic; animal did not react to handling of vagi. Completed at 17.10.

18.10. 5 c.c. of sticky duodenal secretion.
19.00. 3 c.c. of sticky duodenal secretion.
20.00. 3 c.c. of sticky duodenal secretion.

The seat of the inhibitory effect is below the third thoracic segment of the cord, for there was no secretion during 2 hours after cutting the neural axis of a decerebrate cat at the atlanto-occipital membrane and at the level of the third cervical vertebra, but division of the splanchnic nerves at the end of that period gave rise to secretion immediately.

The Effect of Stimulating the Splanchnic Nerves on the "Paralytic" Secretion.—When the rate of this secretion had become constant the splanchnic nerves were stimulated in the same way as the vagus nerves, a record of blood-pressure being taken from the femoral artery. Stimulation caused a rise of blood-pressure lasting slightly longer than the period of stimulation (due probably to the liberation of adrenalin from the suprarenal glands). The blood-pressure in the intervals between stimulation was somewhat above the initial resting level. As long as the stimulation was continued the rate of the "denervation" secretion by the duodenum was considerably diminished in all experiments. For example:

Cat 137. Male, 2·9 kg.

10.45. Ether anæsthesia. Chest opened and splanchnics cut and placed on electrodes. Common bile duct, accessory pancreatic and pancreatic ducts tied. Obturator placed in duodenum 6 cm. from pylorus and retrograde cannula passed through pylorus to drain duodenum. Stomach cannulated. Small gut cannulated 15 cm. below obturator in duodenum. Decerebration completed 12 noon.

14.00. Some secretion.

16.00.	14 c.c. of watery gastric juice.
	9 c.c. of duodenal juice.
	5 c.c. of jejunal juice.
17.20.	6 c.c. of gastric juice.
	6·5 c.c. of duodenal juice.
	3 c.c. of jejunal juice.
	Stimulus started.
19.00.	2 c.c. of watery gastric juice.
	1·5 c.c. of duodenal juice.
	Nothing from jejunum.
	Stimulus stopped.
20.20.	2 c.c. of gastric juice.
	7 c.c. of duodenal juice.
	1 c.c. from jejunum.

An attempt was made to investigate the nature of this inhibition by abolishing the vasomotor effect by ergotoxine.[1] As soon as the rate of secretion had become constant the animal was given ergotoxine (about 2 mg. of ergotoxine ethanesulphonate in divided doses) which abolished the response of the blood-vessels to adrenalin or sympathetic stimulation. Even when the blood-pressure remained at a high level following the ergotoxine secretion stopped. This made it impossible to elucidate the question further by this means.

Effect of an Anæsthetic.—Sodium luminal (150 mg. per kilo intramuscularly) reduced the rate of the "paralytic" secretion almost to zero in a decapitate cat, though the anal reflex was not abolished. Eserine (2 doses of 0·6 mg. at 20 minutes' interval) improved the rate of secretion under this narcosis.

Atropine.—Atropine (1 c.c. of 1 p.c. atropine sulphate intravenously) caused a cessation of this "paralytic" secretion for at least 3 hours.

Character of Fluid.—The fluid secreted had all the physical characters of that obtained by vagus stimulation, was chemically identical (so far as tested) and gave similar results to enzyme tests (see later).

The Relation of the Splanchnics to the Vagus.—It was thought that with the splanchnics cut, the small intestine other than the duodenum might respond to stimulation of the vagus.

Animals were prepared with the vagus nerves on electrodes, the intestines were cannulated as described and the animals were then decerebrated. Two hours later the chest was reopened without anæsthesia and the splanchnic nerves were divided. Two hours were allowed for the rate of "paralytic" secretion to be determined. In two experiments the rate of secretion from the duodenum was greatly

[1] We are indebted to Professor J. H. Burn for assistance and advice in conducting these experiments.

accelerated when the vagus was stimulated, while in one a meagre and in the other a copious flow of juice began from the small intestine. This intestinal secretion ceased when stimulation of the vagus ceased. The procedure of opening the chest a second time, and especially the handling of the splanchnics without anæsthesia, was severe and only these two animals lived long enough to give a satisfactory demonstration of the effect. For example:

Cat 152. Female, 2·8 kg.

10.45. Ether anæsthesia. Chest opened. Vagi placed on electrodes. Chest closed. Common bile duct, accessory pancreatic duct and pancreatic duct tied and cannulæ placed. Decerebration 11.45.

14.50. Chest reopened and sympathetics divided. 5 c.c. gastric juice had collected. Duodenal juice appeared at once.

16.50. 5 c.c. gastric juice.
3 c.c. duodenal juice.
No jejunal juice.
Stimulation of vagi begun.

18.10. 35 c.c. gastric juice.
12 c.c. duodenal juice, almost watery at the end of the period.
14 c.c. turbid, yellowish jejunal juice.
Stimulus stopped.

20.00. Stomach, 5 c.c.
Duodenum, 2 c.c.
No further secretion from jejunum.

From the foregoing experiments it is clear that the greater splanchnic nerve carries inhibitory fibres to the duodenum and possibly to the upper jejunum. The inhibitory centre is probably in the spinal cord. The integrity of the vagi is immaterial to the secretion of "paralytic" juice. "Paralytic" juice from the duodenum is identical in appearance with that produced by vagal stimulation, and as it contains Brunner's gland mucin cannot be looked on as a simple transudate.

As the greater splanchnic only carries inhibitory fibres to a small section of the upper part of the intestine, the possibility of the passage of inhibitory fibres to the cœliac ganglia by other nerves was investigated.

In the first place the experiment of Moreau was repeated, *i.e.* the effect was observed of the removal of the nerves accompanying the intestinal vessels to a loop of small intestine.

In most of such experiments a clear-cut confirmation of Moreau's results was obtained, *e.g.*:

16.00.	14 c.c. of watery gastric juice.
	9 c.c. of duodenal juice.
	5 c.c. of jejunal juice.
17.20.	6 c.c. of gastric juice.
	6·5 c.c. of duodenal juice.
	3 c.c. of jejunal juice.
	Stimulus started.
19.00.	2 c.c. of watery gastric juice.
	1·5 c.c. of duodenal juice.
	Nothing from jejunum.
	Stimulus stopped.
20.20.	2 c.c. of gastric juice.
	7 c.c. of duodenal juice.
	1 c.c. from jejunum.

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Atropine.—Atropine (1 c.c. of 1 p.c. atropine sulphate intravenously) caused a cessation of this "paralytic" secretion for at least 3 hours.

Character of Fluid.—The fluid secreted had all the physical characters of that obtained by vagus stimulation, was chemically identical (so far as tested) and gave similar results to enzyme tests (see later).

The Relation of the Splanchnics to the Vagus.—It was thought that with the splanchnics cut, the small intestine other than the duodenum might respond to stimulation of the vagus.

Animals were prepared with the vagus nerves on electrodes, the intestines were cannulated as described and the animals were then decerebrated. Two hours later the chest was reopened without anæsthesia and the splanchnic nerves were divided. Two hours were allowed for the rate of "paralytic" secretion to be determined. In two experiments the rate of secretion from the duodenum was greatly

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16.10. 4·0 c.c. of duodenal juice.
Only a few drops of juice from ileum, but intestines look full.
Peristalsis still active.
17·40. 5·0 c.c. of duodenal juice.
1 c.c. of juice from ileum. Peristalsis now slight.
17.45. Killed. Intestines removed and emptied.
A total of 21·3 c.c. of juice was collected from the duodenum and 30 c.c. from the rest of the small intestine. The stomach contained several c.c. of thick mucoid acid secretion.

In one animal the greater and lesser splanchnics only were cut. This gave secretion from both the duodenal and ileal cannulæ, but the quantity was less than in those experiments in which all preganglionic fibres were cut.

In two control cats in which the abdominal contents were displaced as if for cutting the nerves and received considerable handling no juice was obtained from the small intestine.

Action of Ganglia at Root of Mesentery.—The possibility existed that the cells of the cœliac ganglia might themselves exert some inhibitory action, *via* the mesenteric nerves.

At aseptic operation all preganglionic fibres and a portion of the abdominal sympathetic chain were removed transperitoneally. For 2 to 5 days following this the cats passed loose motions, but thereafter behaved as normal animals. Some 10 to 14 days later they were decapitated, the intestines were prepared as before described and the ganglia surrounding the mesenteric artery were then removed. These cats gave quite remarkable responses. In the first cat 4·8 c.c. of duodenal and 28·3 c.c. of ileal juice were produced in 3½ hours, in the second 7·6 c.c. of duodenal and 48·4 of ileal juice in 4 hours, in the third 5·7 c.c. duodenal and 22·6 c.c. ileal juice in 2½ hours and in the fourth 3·0 c.c. duodenal and 20 c.c. ileal juice in 4 hours.

The following protocol shows that juice did not flow till the ganglia were removed:—

Cat 238*a*.

6th March 1939. Ether. Aseptic operation.
Midline abdominal incision. Gut, etc., pushed to one side and splanchnics and all visible branches going to cœliac ganglia cut. Portions of abdominal sympathetic chain with ganglia taken out. Abdomen sewn up with fine silk.
8th March. In good condition. No diarrhœa.
11th March. Took only milk, refused meat. Some diarrhœa.
16th March. In good condition, has been eating well, no diarrhœa.
Has been starved for the last 24 hours.

Cat 212.

9.15. Decapitated.
12.45. Two loops of lower ileum were tied off and one denervated.
17.00. Abdomen reopened. Denervated loop was full of secretion. Innervated loop was empty.
18.30. Animal killed.

From the denervated loop, which was about 7 cm. long, 9 c.c. of fluid were collected; the fluid was turbid and contained much mucus. The control loop was moist, with little secretion; there were many tape worms in it.

Occasionally, however, secretion collected in loops whose innervation had not been interfered with (this happened three times in seventeen experiments). The reason for this is not clear.

To investigate the matter further, preparations were made in which all the preganglionic fibres to the cœliac ganglia were cut. The decapitate animals were prepared as follows:—

Through a midline abdominal incision the pancreatic ducts and bile duct were tied. A submucous ligature was passed and tied at the pyloric sphincter and cannulæ were inserted about 8 cm. down the duodenum and into the terminal ileum. In some experiments a cannula was also put in about half-way down the small intestine. The preganglionic fibres were then cut transperitoneally, in some animals after a control period of 2–3 hours, in others at the same time as the preparation of the intestines. It was found in general in this series of experiments that those animals secreted best which were denervated when the preparation was "fresh."

Good secretion was obtained in 6 cats. In 5, more juice was obtained from the jejunum and ileum than from the duodenum. In one animal 49·5 c.c. of duodenal juice and 19·1 c.c. of ileal juice were collected in $5\frac{1}{2}$ hours. Details of an experiment are as follows:—

Cat 224.

11.00. Decapitated.
11.40. Pancreatic, accessory pancreatic and bile ducts tied.
Submucous ligature tied at pylorus.
All preganglionic nerves cut on both sides. Cannulæ tied into lower end of duodenum and terminal ileum. Abdominal window sewn in.
12.00. Operation finished.
14.15. 7·5 c.c. sticky duodenal juice.
7·5 c.c. sticky juice from ileum.
Since beginning of experiment very active peristalsis of small gut and cæcum. Small gut appears to contain a considerable amount of secretion.
15.20. 4·8 c.c. juice from duodenum.
18·0 c.c. juice from ileum.

occurred. The eserine was effective whether or not the vagus nerve had been cut below the lung root. Atropine stopped its effect and sodium luminal (150 mg. per kilo) nearly did so. In no case in which eserine produced a flow of intestinal juice was there any secretion of sweat on the pads of the feet, of mucus in the trachea or of saliva.

The effect of eserine is shown in the following protocol:—

Cat 108. Female, 3·00 kg.

12.30. Ether anæsthesia.

Bile and accessory pancreatic ducts tied, pancreatic duct cannulated. Submucous ligature tied at pylorus, duodenum cannulated and a loop of small gut 15 cm. long tied off and cannulated.

14.20. 0·6 mg. eserine *subcutem*.

14.40. 0·6 mg. eserine *subcutem*.

15.00. 0·6 mg. eserine *subcutem*.

16.42. 0·6 mg. eserine *subcutem*.

17.00. From stomach 2·1 c.c. of juice.

From duodenum 6·5 c.c. of sticky juice, 1 c.c. neutralises 0·3 c.c. N/10 HCl; the mucus is not precipitated by adding acid.

From ileum 22·0 c.c. of slightly sticky juice, 1 c.c. neutralises 0·15 c.c. N/10 HCl; the mucus is precipitated by adding acid.

18.05. Drop or two from stomach.

4·3 c.c. duodenal juice.

0·8 c.c. ileal juice.

18.35. 0·6 mg. eserine.

19.05. 4·1 c.c. duodenal juice.

12·0 c.c. ileal juice from intestinal loop (from which tip of cannula had come out).

The effect of eserine was also tested on a cat bearing a duodenal fistula. A dose of 0·6 mg. was given *subcutem* to this animal after it had been starved to abolish secretion from the fistula. The animal rapidly showed signs of excitement, inco-ordination and itching. Secretion soon appeared at the mouth of the fistula and continued for an hour. The excitement of the animal prevented collection of the secretion.

The secretion produced in the duodenum by eserine was sticky and almost clear and had the characteristics described for the juice produced by vagal stimulation. That from the small gut differed in being watery with no stickiness whatever, and even the last portion of as much as 35 c.c. of secretion collected from a small loop was turbid from cells floating in it. It was just alkaline to litmus and had no significant neutralising value.

Acetylcholine was given *subcutem*, after eserinisation, to decerebrate

12.00 noon. Decapitated. Duodenum and lower ileum cannulated. Main pancreatic and bile ducts tied. Submucous ligature tied at pylorus.
14.00. Few drops of juice present in both cannulæ. Ganglia removed.
15.20. Duodenum 1·8 c.c.
Rest of small gut 6·2 c.c.
16.30. Duodenum 3·2 c.c.
Small gut 4·8 c.c.
17.10. Small gut 16·3 c.c.
17.25. Small gut 8·8 c.c.
18.00. Duodenum 2·6 c.c.
Small gut 5·1 c.c.
11·0 c.c. clear juice in stomach.

The character of the intestinal juice varied considerably. In some cases it contained much thick mucus mixed with opaque white material. In others, especially the later portions to be collected, the juice was fluid, though usually somewhat turbid. The duodenal juice always preserved the physical characters already described, but it also was turbid.

From all the experiments on "paralytic" secretion, it can be said that cutting the greater splanchnic in the thorax "releases" the duodenum only and that cutting all preganglionic fibres "releases" the whole of the small intestine; if recovery is allowed after cutting the preganglionic fibres the cœliac ganglia take over an inhibitory rôle, as shown by the free secretion which occurs if they are later removed.

The Effects of Eserine and Acetylcholine.

As a preliminary to testing the effects of acetylcholine, eserine was given to decerebrate animals with the intestines cannulated as usual, but with the nerve supply intact. In some cats, instead of the usual two doses of 0·6 mg. of eserine *subcutem* at 20 minutes' interval, three or even four doses of this amount were given at the same interval. While waiting for the drug to take effect before giving acetylcholine we were surprised to see secretion appear from the cannulæ in the small intestine and duodenum. This observation was frequently confirmed. Quite large quantities of juice could be collected from both portions of the intestine. The amount of eserine necessary to produce this effect was somewhat variable; in some cats secretion began after only two doses of 0·6 mg., and in no cat were more than four doses necessary. Similarly, there was great variation from animal to animal in the frequency with which it was necessary to repeat the administration of eserine to keep the flow going. In no case was there a failure of secretion from the duodenum, but in some cases (two out of seven experiments) there was a failure of secretion from the small intestine other than the duodenum. No significant increase of secretion from the stomach

secreted "normal" intestinal juice, presumably in response to mechanical stimulation. Florey and Harding [1935 *a* and *b*] transplanted the first part of the duodenum subcutaneously in cats and showed that such denervated fistulæ responded to feeding after starvation by secreting a clear mucoid juice; no local or artificial stimulation of any sort was used. The same fistulæ secreted *in response to an intravenous injection* of highly purified secretin. Nasset *et al.* [1935], Nasset [1938], and Nasset *et al.* [1938] performed experiments demonstrating the possibility of a humoral stimulus to intestinal secretion, but they doubted whether secretin was the hormone concerned. They described a new hormone, enterocrinin, which they believed was the substance involved.

The following experiments on the influence of hormones on secretion were performed exclusively on that part of the intestine containing Brunner's glands.

Cats.—We have repeated the transplantation of the duodenum on four cats with results which agree with those previously reported. There can be no reasonable doubt that some humoral mechanism exists in the cat for the activation of the upper part of the duodenum. Intravenous secretin caused secretion from these fistulæ.

Pigs.—The humoral control of duodenal secretion has also been investigated in the pig. Transplantation methods were again used. A portion of duodenum from between the bile and pancreatic ducts was isolated [Goodfriend *et al.*, 1938] and placed subcutaneously while still retaining its mesenteric pedicle, in the way already described for the cat and rabbit [Florey and Harding, 1935 *a*]. Six weeks later the pedicle was cut, thus isolating the fistula beneath the skin of the abdominal wall.

Three pigs were operated on in this way, and in all the fistulæ have secreted in response to food, even on the day following the cutting of the pedicle. The following protocol shows the amounts of secretion obtained in one of these pigs with a subcutaneous fistula:—

The pig was starved for 24 hours, but was allowed water. The secretion was then collected.

1st hour, 0·8 c.c.
2nd hour, 0·1 c.c.
3rd hour, 0·0 c.c.
4th hour, 0·0 c.c.

The pig was then fed.

1st hour after feeding, 9·8 c.c.
2nd hour after feeding, 13·1 c.c.
3rd hour after feeding, 13·0 c.c.

The secretion was typical mucoid duodenal juice.

Thus in the pig as well as the cat the duodenum secretes a mucoid fluid in response to food after all extrinsic nervous influences have been removed.

cats of which the intestines had been cannulated. The maximum safe dose for animals which had been prepared as described and had been left for 2 hours to avoid anæsthetic effects was 2 mg. This dose gave a definite increase in the rate of secretion from the small intestine, or started secretion if not already present, and gave a slight increase in gastric secretion. When, however, a full rate of secretion had been excited by four doses of 0·6 mg. eserine, acetylcholine did not increase the rate of secretion. The intestinal juice changed somewhat in character after giving acetylcholine. The change ranged from an increase in the turbidity of the juice, through an intermediate stage with "clots" of cast-off villous epithelium, to a juice in which villi and some blood were present. At the post-mortem examination the intestines which had shed villi showed localised hæmorrhagic patches in the mucosa up to 1 cm. in diameter from which the villi both macroscopically and microscopically were gone. This effect was inhibited by atropine sulphate (1 c.c. of a 1 per cent. solution given intravenously).

Influence of Hormones on Intestinal Secretion.

Delezenne and Frouin [1904] were the first to report an abundant secretion from a fistula in response to the intravenous injection of secretin-containing preparations. Their fistula was made from the duodenum (below the pancreatic duct) and upper jejunum of a dog. Bottazzi and Gabrieli [1905–06], in acute experiments on dogs, found that large injections (15–40 c.c.) of duodenal extracts produced a secretion of clear and slightly yellow juice which sometimes contained mucus and debris; they thought the active substance was a "secretin" similar to pancreatic secretin. Foà [1908] could not always obtain a secretion by Bottazzi and Gabrieli's method, and many dogs were killed by the injections. Brynk [1911] failed to confirm Delezenne and Frouin when he put 200–300 c.c. of HCl of different strengths into the stomach and got no increased secretion from a Thiry-Vella fistula. Delezenne and Frouin had obtained a secretion by this procedure, though they were clear that the stimulus came from the intestine, not from the stomach. Frouin [1905] found that the intravenous injection of *succus entericus* itself caused intestinal secretion; his result is possibly explained by the observation of Volborth [1925] that the deposit from intestinal juice contains a "secretin" which stimulates the pancreas (though Frouin noticed no effect on the pancreas from his injections). Ågren [1934] reported that injections of highly purified secretin products caused intestinal secretion, though his results are perhaps not very clear cut. Authors have also reported stimulation of intestinal secretion by a variety of apparently non-specific substances [Mironescu, 1909; Hirata, 1910; Komarov, 1924 *a* and *b*, 1926]. Ivy *et al.* [1927] noted incidentally that loops of intestine transplanted beneath the skin

The pig was starved for 24 hours, but allowed water. The secretion was then collected.

1st hour, 1·2 c.c.
2nd hour, 0·3 c.c.
3rd hour, 0·0 c.c.
4th hour, 0·0 c.c.

The pig was then fed.

1st hour after feeding, 15·8 c.c.
2nd hour after feeding, 17·3 c.c.
4th hour after feeding, 17·0 c.c.

Dogs.—Some observations on the effects of starvation and of secretin injections were also made on upper duodenal fistulæ in dogs. Ponomarew [Babkin, 1928] worked with dogs and appears to have been the first to prepare fistulæ of that part of the intestine containing Brunner's glands. His preparations (2 dogs) gave a small flow of mucoid alkaline juice which appeared to be uninfluenced by starvation. Ponomarew's fistulæ were presumably innervated by the vagus, as he closed the pylorus by a submucous ligature leaving the muscle intact. Florey and Harding [1934] made fistulæ in 2 dogs in which the stomach was cut right across at the pylorus; these fistulæ were therefore partially denervated. An alkaline mucoid juice was obtained—about 1·5 c.c. per hour—the flow of which was reduced but not abolished by 24 hours' starvation.

In the present work difficulties were encountered in preparing the fistula in one operation. Although the surgical procedures appeared to be adequate the first three dogs died 3, 5, and 11 days after operation. There was nothing obvious to account for these deaths as the dogs were not greatly shocked after operation and appeared to do well until they died quite suddenly. By adopting the following two-stage procedure we prepared two very satisfactory animals.

At the first operation the mucosa of the stomach just proximal to the pylorus was separated from the muscle by blunt dissection through an incision placed longitudinally in the gastric muscle near the greater curvature. In this way a minimum of disturbance to the nerves passing from the stomach to the duodenum was ensured. The mucosa was then cut across and the proximal end insewn; the distal end was removed as far as the pyloric ring, which was then closed by sutures. The incision in the gastric muscle was sewn up and a gastro-enterostomy was made to the first part of the jejunum. The abdomen was sewn up and the animal allowed to recover for 14 days.

At the second operation the duodenum was cut across just above the bile duct and the distal end was insewn, avoiding compression of the bile duct. The proximal cut end was brought to the surface in the upper part of the midline abdominal incision and sewn to the muscle

Fully innervated fistulæ of the pig's duodenum have been made for comparison with the foregoing isolated fistulæ. As this type of fistula has not been prepared before in the pig a description of the method is given.

A young pig (usually weighing about 12 kg.) was starved for 24 hours before operation. Open ether anæsthesia was used. The pyloric end of the stomach was brought out through a midline incision. A cut was made through the muscle, parallel to the long axis of the pylorus, and the mucosa was dissected off the muscle all round the pylorus with as little disturbance to the muscle as possible. The mucosa was then cut through, the upper cut end was insewn and the lower part was excised as far as the beginning of duodenal mucosa. The bared gastric muscle was brought together by a few loose stitches and the longitudinal cut in the muscle was sewn up. In this way the lumen of the duodenum was separated from that of the stomach, while leaving the nervous pathways in the muscle and along the vessels intact. Next the bile duct was detached from the duodenum; in some cases a piece of duodenal mucosa round the entrance of the duct was excised, in others the duct was cut across where it entered the intestinal muscle. The hole left in the duodenum was sewn up. The duodenum was cut across above the pancreatic duct. The lower cut end was insewn and the bile duct was anastomosed to this part of the duodenum near the suture-line. If the papilla had been excised with the duct the mucosa surrounding it was sewn into a small hole in the duodenum. If the duct had been cut across the best procedure was found to be to tie a glass cannula into the free end of the duct; a short piece of rubber tubing was slipped on to the cannula to make subsequent manipulations easier. A small hole was made in the duodenum through which the rubber tube was pushed, followed by the glass cannula and the cut end of the duct. A purse string suture previously placed around the hole in the gut was tied round the duct and cannula and, to hold it more firmly, the duct was sutured into an appropriately placed gutter made by infolding the walls of the duodenum. We consider this procedure easier than transplanting the papilla of the bile duct. A gastro-enterostomy was made to a convenient loop of intestine. The upper cut end of the duodenum was brought to the midline abdominal incision to form the opening of the innervated duodenal fistula. Animals so operated have been kept in health for months after operation. Three were killed for reasons unconnected with the fistula at 2, 6, and 7 months after operation; one is in good condition at the time of writing, 8½ months after operation.

Such a fistula secretes considerable quantities of mucoid juice, exactly similar in physical appearance to that secreted by a totally denervated fistula. These fistulæ also react to starvation and feeding in the same way as the denervated fistulæ; for example:—

occurred. When the secretin was given during stimulation of the vagus there was similarly no increase in alkilinity.[1]

Effects of Histamine.—Koskowski [1926] stated that subcutaneous histamine injections caused a secretion of intestinal juice from a Thiry-Vella fistula in dogs. He did not, however, give any figures of the quantities obtained. Nechoroschew [1929], using dogs with fistulæ and decerebrated cats and dogs, also concluded that histamine given enterally caused an increase in the motor and secretory activity of the intestine. From his description it is difficult to know exactly how his experiments were done and the amounts of intestinal secretion reported were very small. Cajori [1933] examined histamine juice produced from dogs reported on later by Berndt and Ravdin [1934]. These authors used a modified Thiry loop with Johnson's [1932–33] catheter. They noticed a considerable increase in the amount of secretion following the subcutaneous administration of histamine. The increase reached its maximum half an hour after the injection. On the contrary Lim *et al.* [1922–23] found no evidence of a secretory effect on the human duodenum. Florey and Harding [1934], from experiments on decerebrate cats, concluded that histamine did not stimulate the secretion of Brunner's glands.

In the decerebrate cat, in the present series of experiments, we were not able to collect any secretion from the duodenum after 3·5 mg. histamine phosphate, given subcutaneously in divided doses. The stomach secreted 85 c.c. of watery acid juice during an experiment which lasted 4 hours, but no fluid appeared in the duodenum. The administration of eserine then gave a copious flow of duodenal secretion, showing that the failure of secretion was not due to any fault in the preparation.

It is possible that some of the positive effects reported were due to the liberation of acid gastric juice which passed into the intestine to liberate a hormone acting on the intestinal mucosa (*cf.* section on hormone stimulation).

Histological Investigations.

Stimulation of the vagi causes great depletion of the mucin contained in Brunner's glands; in some cases almost complete exhaustion occurs (figs. 1 and 2).

The effect on the crypts of Lieberkühn was in some experiments negligible, but in others there was flattening of the epithelial cells with apparent dilatation of the lumina of the crypts, in which there was coagulated material.

Brunner's glands also showed evidence of exhaustion following section of the splanchnics, and in some specimens there was œdema of

[1] The late Professor J. Mellanby very kindly supplied the secretin used in these experiments.

and skin. The animals were fed on milk, syrup and crushed biscuit, with meat after the first 3 days. They are alive and well 2½ and 3 months respectively after the second operation. The fistulæ secrete a clear mucoid juice which has floating in it small white flecks of débris some of which are difficult to centrifuge out. The juice is either colourless or faintly brown. The following are some figures illustrating the effects of 24 hours' starvation and subsequent feeding:—

After 24 hours' starvation (water allowed) a pot was tied over the opening of the fistula.

1st hour, 0·0 c.c.
2nd hour, 0·0 c.c., fistula completely dry.
3rd hour, 0·0 c.c., fistula completely dry.

The dog was then fed.

1st hour after feeding, 0·9 c.c.
2nd hour after feeding, 1·3 c.c.
3rd hour after feeding, 1·5 c.c.

The other dog gave a similar result and secreted 1·6, 2·1, 2·0 and 2·0 c.c. of juice in the first 4 hours after feeding. The fistulæ in these dogs therefore reacted to starvation in the same way as those in cats and pigs, and gave decisive evidence that a flow of juice is produced by feeding in this species.

Injections of crude secretin were also given:

Dog 1. The dog was starved as before. In the two hours before the injection no juice could be collected. 2 mg. of secretin were injected intravenously and in the next half-hour 0·8 c.c. of typical sticky duodenal juice was collected. This rate was approximately the same as that after giving food.

Dog 2 was also starved. No juice could be collected before the injection and the mucosa looked dry. 2·5 mg. of secretin were injected and secretion was seen inside the fistula within 2 minutes. About 0·5 c.c. was collected in the 15 minutes after the injection, but as the pot slipped an exact measurement was not obtained.

Acute Experiments.—The evidence for a humoral control of the duodenal secretion received support from experiments done with decerebrate cats. To test the effect of secretin the intestine and pancreatic duct were cannulated in the usual way. A dose of secretin which gave 2 c.c. of pancreatic juice gave 0·3 to 0·5 c.c. of tenacious mucoid secretion from the duodenum. Similar secretion occurred each time the dose was repeated.

It was thought that the secretin might affect the Brunner's glands in the same way as the pancreas, exciting an outpouring of alkali as well as mucus. A free flow of eserine juice was excited before the secretin was given to animals prepared as usual. The alkalinity of the juice was compared before and after the secretin; no significant increase

FIG. 2—Alveoli of Brunner's glands exhausted of their mucin following vagal stimulation. Note wide lumina of alveoli, traces of darkly stained mucin at the free borders of the alveolar cells and the rounded clearly seen nuclei each with its nucleolus.

PART II. ENZYME INVESTIGATIONS.

INTRODUCTION.

Duodenal Juice.—Little work has been done on the digestive function of juice from the Brunner's gland area, because in all species it is a small part of the intestine and relatively difficult of access. Colin [1854] collected duodenal juice from the horse and accurately described its physical characters. Kuhne [1868] stated that the Brunner's gland area gave a viscous extract which did not digest fat, and Brown and Heron [1879–80] found that this distinctive extract had the most feeble digestive power of any extract from the small intestine. The St. Petersburg school (working entirely, so far as is known, with dogs) reported that duodenal juice contained pepsin and rennin-like enzymes [Ponomarew, 1902, quoted by Babkin, 1928; Pawlow and Parastschuk,

the tips of the villi. Eserine also produced histological evidence of activity of Brunner's glands in some cases, but when acetylcholine was also administered great exhaustion of these structures was apparent. In nearly all experiments using acetylcholine, however, there was

FIG. 1.—Alveoli of Brunner's glands from a cat starved during the previous 24 hours. Mucin (stained with gentian violet) can be seen to fill the alveolar cells, the nuclei of which are flattened towards the cell-base.

damage to the mucosa varying from slight hæmorrhages in the villi to desquamation and destruction of the superficial mucosa.

In the Moreau-type experiment the appearances were variable. In some there was considerable destruction of the surface epithelium, while in others, even if the secretion had been good, no such thing occurred. In some cases there was flattening of the epithelial cells of the crypts, while the villi retained their normal appearance.

To sum up, such histological observations as have been made fully bear out the experimental data concerning the activity of Brunner's glands, but the present collection of material does not give any clear-cut or consistent histological picture of the effects of the various procedures on the other intestinal structures.

FIG. 2.—Alveoli of Brunner's glands exhausted of their mucin following vagal stimulation. Note wide lumina of alveoli, traces of darkly stained mucin at the free borders of the alveolar cells and the rounded clearly seen nuclei each with its nucleolus.

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1904] and a feeble or inconstant amylase, invertase, and lipase [Ponomarew]. Abderhalden and Rona [1906] confirmed the pepsin-like enzyme. Pavlov [1910] said that trypsin was chiefly activated by the duodenal secretion. In 1934 Florey and Harding, using rather crude methods, tested for enzymes in juice from duodenal fistulæ in the goat, pig, dog, cat and rabbit. They found a pepsin-like enzyme in the goat and dog, and a trace of lipase in the dog.

Juice from the Jejunum and Ileum.—In contrast with duodenal juice, the *succus entericus* from the rest of the small intestine has often been investigated. Frerichs in 1846 claimed to be the first to tie off an empty loop of small intestine and collect and describe the juice which accumulated in it. The same method was used by Colin [1854], but was superseded in 1864 by Thiry's operation for making a permanent fistula, subsequently modified by Vella [1888].

A great many experiments were reported in the fifty years after Thiry described his operation, culminating in a spate of work from the St. Petersburg school after 1899, summarised by Pavlov in *The Work of the Digestive Glands* [1910]. Chemical methods were primitive and possible fallacies often not recognised, so nothing is to be gained by reviewing the work of this period in detail; there were many negative results, but the enzymes reported present by one or another worker included amylase, invertase, maltase, lactase, arginase and trehalase, splitters of fibrin, egg albumen, meat and casein, a rennin, a lipase, a fat emulsifier, splitters of peptone and peptides (erepsin), and enterokinase.

Recent workers have also found a variety of enzymes. Orbéli and Sawitch [1917] found in human intestinal juice amylase, invertase and maltase, lipase, erepsin and enterokinase, but no lactase. In human ileal juice, Hermann and Ribère [1931] found no enzyme digesting egg-white, a doubtful lipase, a strong invertase, feeble amylase and enterokinase, and an erepsin which was strong during digestion and weak or absent in starvation. Bickel and Kanitz [1934] found amylase and erepsin, but no lipase, in mucus-free samples of uncentrifuged human juice.

In dog intestinal juice Roger and Binet [1921] found lipase, le Breton and Mocoroa [1931] found peptidase and enterokinase but no trypsin-like enzyme, and Koskowski [1926] found amylase and invertase, but described them as endo- (*i.e.* cell) enzymes. Schwichtenberg and Burget [1931–32], using juice from closed intestinal loops, found invertase in 75 per cent. of jejunal and 50 per cent. of ileal samples, and lipase occasionally. Pierce *et al.* [1935], who macerated the deposit with the supernatant juice, found amylase, invertase, maltase, lactase, erepsin and a feeble lipase; casein and albumen were not digested at pH 6, but gelatine was slightly digested when bile was present. Cajori [1933] found feeble erepsin and invertase, which he thought came

from ruptured cells, and a stronger amylase and [1935] traces of lactase.

Babkin [1928], in his review of intestinal secretion, stated that feeble amylase and lipase, stronger invertase, maltase and lactase and also nuclease, erepsin and enterokinase were present in the juice.

Sources of Reported Enzymes.

Intestinal juice consists of a secreted fluid together with intestinal bacteria and cast-off cells, so that beside the secretion possible sources of enzymes are bacteria and ruptured cells. Traces of gastric and pancreatic enzymes adhering to the mucosa could be found in acute or recent preparations.

Leube [1868] found that filtered juice did not always digest cane-sugar, whereas the whole juice did so, and he was the first to suggest that the enzymes originated in the "Flocken" and passed from them into the fluid part of the juice.

The "Flocken" of the German literature were floccules of mucus mixed with opaque matter which sank in the juice; they were present in juice obtained by a variety of methods of stimulation. As an extreme instance, Masloff [1882] reported a mass occupying a third of the volume of the juice. Microscopic examination showed the floccules to contain "mucous cells" [Masloff], round cells [Dobroslawin, 1870], leucocytes [Gumilewski, 1886], "fat" from cell debris [Röhmann, 1887], and coccoid bacteria [Pregl, 1895]. The cells were usually very degenerate. Hermann and Ribère [1931] found leucocytes, desquamated epithelial cells and mucus in juice from a human fistula. Even in relatively clear juice a small deposit can usually be obtained on centrifuging and shown to consist of amorphous material containing bacteria and a few still recognisable cells [Florey and Harding, 1934]. Kühne [1868] was one of the few workers who described juice from a fistula as being clear and free from floccules.

Ramond [1904] observed that mucosal cells and leucocytes were shed from the mucosa of the intestine during digestion. In the fistula, peristalsis and the normal growth of the mucosa may similarly cause cells to be shed into the juice, while partial obstruction or slight sepsis at suture-lines would account for the presence of polymorphs and of non-intestinal bacteria. Leube found that solid matter accumulated in the fistula during starvation, when no fluid was being secreted.

(a) *Cell Enzymes.*—No one disputes that the epithelial cells of the intestinal mucosa contain enzymes which can split food substances, for example, peptidase (erepsin) [Cohnheim, 1901] and invertase [Paschutin, 1871; Bernard, 1873]. Less is known about the enzymes of round cells and polymorphs. A number of workers have compared turbid

or mucoid juice or centrifuged deposit, which would all be likely to contain cell debris, with clear or fluid juice.

Kühne [1868] compared clear fistula juice, which did not digest starch, fat, albumen or meat, with juice obtained by other workers from tied-off loops which, owing, he believed, to pancreatic enzymes or to solid matter, digested those substrates. Tubby and Manning [1892] noticed that starch and cane-sugar were more often digested by the mucous than by the fluid part of the juice from a human fistula. Frouin [1906] found saponification of fats by whole juice from dog fistulæ, and by the centrifuged deposit, but not by centrifuged and filtered juice. Bierry and Frouin [1906] found that at the height of secretion dog fistula juice was turbid owing, they believed, to cell débris separated from the fistula by peristalsis. By first washing out the fistula they were able to collect clear juice. Both turbid and clear juice were centrifuged and passed through a Berkefeld filter. The clear juice digested maltose, not starch or cane-sugar; the turbid juice digested maltose, starch, cane-sugar and trehalose; extracts of intestinal mucosa treated in the same way digested the same four carbohydrates. Jansen [1910] with dog fistulæ found that the mucoid part of the juice was more lipolytic than the supernatant fluid. Koskowski [1926], also working with dogs, compared clear juice, turbid juice, and turbid juice which had been shaken with glass beads to break up the cells; the first two digested starch and cane-sugar feebly, the last strongly.

These all found that the digestive power of turbid or mucoid juice was stronger than that of clear or fluid juice. On the other hand Dobroslawin [1870] found that floccules suspended in water were less amylolytic than filtered juice. Hamburger and Hekma [1902 and 1904] found that both filtered and unfiltered juice digested casein, peptone, starch and cane-sugar, and that both contained enterokinase; their subject was a human patient and it is likely that unfavourable conditions such as a long time or a high temperature during collection might lead to cells breaking up and liberating their enzymes before the juice was filtered; relatively rough chemical tests might then fail to show any difference in the digestive power of the two kinds of juice. The same fallacies may account for the finding of amylase, invertase, erepsin and enterokinase by Hermann and Ribère [1931] in human fistula juice in spite of prolonged and vigorous centrifuging. Foà [1908] took precautions against cell enzymes by centrifuging dog fistula juice as soon as it was collected and later passing it through a bacteriological filter, and it is a pity that his chemical methods do not justify his conclusion that peptone was digested.

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proteins themselves." They adduced no chemical or other evidence for this supposition, but supported it [Nasset *et al.*, 1935] by calculating that 0·5 mm. of mucosa would have to be shed from the surface of a Thiry fistula to produce the amount of solid material in a 6–8-hour collection of juice. Their method of collection, however—passing a catheter to the end of the fistula—may damage the mucosa and rub off many cells.

Nearly all other authors who have considered the point have concluded that some of the enzymes reported in intestinal juice are endo-enzymes from the mucosal cells and not exo-enzymes secreted into the juice. Gamgee [1893] reviewed earlier work and concluded that amylase was in the juice, but invertase was mainly in the cells; Bierry [1912] and Bierry and Frouin [1906] thought that maltase was secreted, but invertase was in the cells; Euler and Svanberg [1921], discussing invertase only, thought that it came from cells and Koskowski [1926] that the amylase and invertase found in the juice were both cell-enzymes. Röhmann and Nagano [1903], working on absorption, concluded that cane-sugar and maltose were split partly in the gut lumen but mainly in the mucosa during absorption, and that lactose was not split by the juice and only slightly in the mucosa. Cajori [1933], who combined investigation of the juice with experiments on absorption from the fistula, found that starch was digested by the juice and absorbed from the fistula at about the same rate, but that the juice secreted in a given time only split a small proportion of the cane-sugar and peptone which could be absorbed from the fistula during the same length of time, and he concluded that certain food materials were mainly digested during their passage through the mucosa. Starling [1911] mentioned that a similar view was held by many physiologists. More recently Howell [1926] wrote: "Experiments have shown that this liquid (the intestinal secretion) has little or no digestive action except upon the starches, and it may perhaps be doubted whether it is a true digestive secretion. Extracts of the walls of the small intestine or the juice squeezed from these walls have been found, on the contrary, to contain four or five different enzymes and to exert a most important influence upon intestinal digestion. These enzymes belong probably to the group of endo-enzymes, and are not actually secreted into the lumen of the intestines. While they are not, strictly speaking, constituents of the intestinal juice, nevertheless it is their action on the food which forms the characteristic contribution to the process of digestion made by the glands of the intestinal wall."

(b) *Bacterial Enzymes.*—Leube [1868] noticed that his digest mixtures became acid and found "vibriones" in them. Kühne [1868] discussed the theory that Pasteur's organisms were responsible for digestion, but argued that higher animals must be as capable of producing chemical ferments as the little "torulaces and vibriones." Masloff

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In addition, certain comparisons have been made. The duodenal juices obtained from acute cat preparations by different stimuli (sympathetic section, administration of escrine, etc.) have been compared with each other and with juices from other levels of the small intestine produced by the same stimuli. The duodenal juices obtained from permanent fistulæ in five different species of animal have also been compared. The distribution, cellular constitution, and staining properties of Brunner's glands vary from species to species [Florey and Harding, 1933, 1934; Carleton, 1935], which justifies a search for differences in the properties of the secretion. Thirdly, a comparison has been made between juice secreted in response to natural hormonal stimuli alone and in response to nervous and hormonal stimuli together, *i.e.* between juice from denervated and innervated fistulæ. Bowie and Vineberg [1935] showed that in the stomach histamine produced a maximal secretion of HCl but no pepsin, whereas stimulation of the vagus produced both acid and pepsin. Pancreatic secretion also varies according to the stimulus applied. It was thought that changes of the same sort might occur in the enzyme content of the duodenal secretion.

Methods.

The methods used for obtaining juice from acute preparations and for making permanent fistulæ have already been described.

Collection of Juice from Permanent Fistulæ.

The duodenal fistulæ, both transplanted and innervated, secreted spontaneously during digestion in all the five species of animal used and the rate of secretion remained constant for each animal for months. Nothing is known about the methods used by Ponomarew and other Russian workers for collecting juice from duodenal fistulæ. The majority of workers on the jejunum and ileum have inserted a catheter into the fistula to drain it and, by mechanical stimulation, to increase the output of fluid. Thiry [1864] and Röhmann [1887] believed fluid obtained in this way to be the normal output of the intestinal glands, but Boldyreff [1928] stated that it differed from spontaneously secreted juice in having weaker enzymes and an inconstant composition, and believed it to be a mixture of exudate and natural juice. Pavlov [1910] believed that a protective secretion of water was produced, as the kinase content of the juice fell rapidly during a period of mechanical stimulation. Mechanically stimulated juice necessarily contains an abnormal number of epithelial cells and sometimes blood.

By tying a small crucible or pyrex pot over the mouth of a duodenal fistula it is possible to collect spontaneously secreted juice without touching or stimulating the mucosa in any way. This method has

[1882] noticed that solution of fibrin only occurred when the smell of the juice indicated decomposition and microscopy showed bacteria.

Many workers used antiseptics to prevent bacterial action. Masloff used thymol and discussed whether it inhibited the enzyme action he was looking for; Mendel [1896] used thymol or sodium fluoride; Widdicombe [1902] found that thymol effectively inhibited bacterial action; Boldyreff [1906–07] used calomel or thymol; Sawitch [1917] used several different antiseptics separately or together and had identical results with all, and Bierry [1912] passed the juice through a Berkefeld filter or used three different antiseptics together. Bastianelli [1892] took more elaborate precautions, washing out the fistula with thymol before collecting the juice and using sterilised vessels; the juice contained floccules and shreds of mucosa and he found that starch and cane-sugar were digested and that egg-white, fibrin and albumose were not. Miura [1895], when investigating the inverting properties of the intestinal mucosa of animals, ingeniously used the mucosa from stillborn infants as a sterile control; he concluded that inversion by the mucosa was independent of bacterial action. Hamburger and Hekma [1902] cultured the juice from their human patient and inoculated the growth into boiled juice and, after incubating, used the culture or its filtrate as a control to fresh juice; they showed that the culture or its filtrate did not activate trypsin, whereas the fresh juice did.

Bacterial enzymes probably caused digestion, as Masloff suggested, in early experiments, when food substances were left in the juice or even inside the fistula for several days and solid pieces of meat, fibrin or coagulated egg-white were found to be "dissolved." But later experiments lasted a shorter time and were done *in vitro*, and those workers who took steps to lessen bacterial action found no difference in the results of the tests.

Summarising the literature, juice from duodenal, jejunal and ileal fistulæ clearly contains a number of enzymes which digest food substances. The enzymes do not appear to be bacterial, but there is considerable evidence that some of them come from autolysed cells and are not secreted with the fluid part of the juice. Possible exceptions are amylase and perhaps maltase; the source of the enterokinase in the juice has not been thoroughly investigated.

Present Work.

The experiments described in the first part of this paper have given the opportunity to investigate the digestive action of duodenal juice obtained by a variety of experimental methods. Since many samples of juice were almost cell free, while others contained many cells, it has been possible to deduce some of the digestive properties of the secreted fluid as distinct from those of the whole juice.

When a few drops or a loopful of the incubated digest at each pH were cultured on agar slopes, the cultures were usually sterile; sometimes they grew one or two colonies. The presence of colonies bore no relation to the occurrence of digestion, and a sterile digest and one which grew a colony gave identical results when digestion was estimated.

Methods of Testing for Enzymes.

The following buffer solutions were used:—

pH 2–8: Sorensen's buffers [Clark, 1928];
i.e. pH 2, 3, and 4, citrate-HCl;
pH 5 and 6, citrate-NaOH;
pH 7, phosphate;
pH 8, borate-HCl;
pH 8 and 9, veronal-HCl [Michaelis, 1930].

The borate buffer was used at pH 8 for ammonia estimations as the veronal buffer was found to give off small amounts of a volatile base after incubation. The veronal buffer was used for all other methods.

1. *Proteases.*—The substrates were 5 p.c. egg albumen, 3 p.c. gelatine and 5 p.c. casein ("soluble" or Hammarsten's casein from Schering-Kahlbaum). Each experiment was set up at pH 2, 4, 6 and 8 or at pH 2, 5, 7 and 9. In a few early experiments digestion was looked for by estimating increases in amino-nitrogen, but the method was abandoned as the increase would be too small if digestion proceeded only to the poly-peptide stage. In subsequent experiments the digest was treated with trichloracetic acid or colloidal iron and increase in non-precipitable nitrogen was estimated by micro-Kjeldahlisation followed by micro-estimation of ammonia [Conway and Byrne, 1933; Conway, 1935].

2. *Cathepsin.*—For activation, the juice (4 c.c.) was incubated with H_2S water (1 c.c.) at 37° C. for half an hour. The H_2S was then removed *in vacuo* at room temperature and the juice was set up for digestion with gelatine and either albumen or casein at pH 4, 6 and 8 or pH 5 and 7. Increase of non-precipitable nitrogen was estimated as above.

3. *Poly- and Di-peptidase.*—The substrates were 5 p.c. "Difco" bacto-peptone and 1·25 p.c., 3 p.c. or 5 p.c. glycyl-glycine (Hoffmann-La Roche). Each experiment was set up at pH 2, 4, 6 and 8 or at pH 2, 5, 7 and 9. The criterion of digestion was increase in amino-nitrogen, estimated by the semi-micro-method of Van Slyke [1913–14].

4. *Amylase.*—The substrate was 1 p.c. boiled potato starch solution. Each experiment was set up at three or four different H-ion concentrations between pH 4 and pH 9. The criterion of digestion was increase in reducing power. Hagedorn and Jensen's [1923] method for estimating reducing substances was used at first, but later was replaced by Miller and Van Slyke's [1936] method, in which ferro-

been used throughout the present work. As soon as possible after collection the juice was centrifuged fast enough to throw down macroscopic particles, and was immediately poured off from the deposit. Then it was shaken with toluol and put in the ice-chest till it was used, usually the next day.

Appearance of Juice.

Acute juices obtained by vagal stimulation, splanchnicotomy and eserinisation were opalescent and had a small deposit. Post-ganglionic sympathectomy juice was turbid with cells. Acetylcholine juice was turbid with cells and often contained whole villi and blood.

Permanent Fistulæ.—Pig juice was usually clear, sometimes faintly turbid, and there was usually a small deposit after centrifuging. Cat and rabbit juice were almost always water-clear and often there was no visible deposit. Goat juice was clear or slightly cloudy. Dog juice more often than the others contained fine floccules; sometimes they came down completely on centrifuging, but sometimes the finest particles remained behind and made the juice faintly turbid. The juice from any animal might occasionally be tinged yellow or brownish, presumably by blood pigments; very occasionally a few red blood corpuscles were present. There was no difference in appearance or consistency between juice from transplanted and innervated fistulæ. Centrifuged deposits consisted of mucus and disintegrating epithelial cells.

Precautions Against Extraneous Enzymes.

1. *Gastric and Pancreatic Enzymes.*—These were removed in all acute experiments by carefully washing out the loop of intestine with saline, or by throwing away the first few c.c. of juice collected.

2. *Cellular Enzymes.*—The first precaution with permanent fistulæ was never to collect juice until the fistula was perfectly healed, the second was not to touch the fistula during the collection so as to avoid rubbing off any mucosal cells, and the third, which applied to acute and permanent preparations, was to centrifuge off as many cells as possible immediately the juice had been collected.

To show the effect of epithelial cells collections of duodenal and jejunal juice were divided into two parts. One part was centrifuged and to the other small amounts of scrapings of intestinal mucosa were added. Tests for di-peptidase and invertase showed that, whereas the centrifuged juices did not digest the substrates, strong digestion occurred in the samples which contained mucosal scrapings.

3. *Bacterial Enzymes.*—The juice was shaken with toluol as soon as it had been centrifuged, unless it was to be used immediately. Toluol was also added to all digest mixtures except in the few lipase tests which only lasted 6 hours. About 4 drops were added to each c.c. of the mixture.

Method of Testing for Lysozyme.

After centrifuging the juice at 5000 r.p.m., serial dilutions were set up against an 18-hour culture of Micrococcus Lysodeikticus suspended in saline, the opacity of the suspension being equivalent to No. 4 Ba sulphate standard. The titre was taken as the highest dilution at which complete lysis, read macroscopically, occurred after incubation for 1 hour at 37° C. Some tests were read again after standing for 12 hours at room temperature. The method is essentially the same as that of Goldsworthy and Florey [1930].

RESULTS.

The tables summarise the enzymes tests on juices obtained by different methods and from different animals. Every test has been entered on the tables, so there is no selection of results. A few qualitative tests which were done at the beginning of the work are included for completeness.

Enzyme Tests on Juices from Acute Preparations (Table I.).

Three complete tests for *protease,* on cat duodenal juices obtained by two different methods, were negative. Three tests on duodenal and two on lower intestinal juice set up at pH 7 only were also negative.

Di-peptidase.—The one test on rabbit duodenal juice was negative. With cat juice, 10 out of 12 duodenal and 2 out of 4 lower intestinal samples were negative. The four juices which gave positive results were exceptionally cellular; one each of these duodenal and lower intestinal samples was obtained by acetylcholine stimulation, which usually brings off villi and fragments of mucosa, and in these particular juices the cellular floccules, instead of being centrifuged out, were chopped to distribute them evenly through the juice, so that it would have been surprising if peptidase had not been present. (The one uncentrifuged acetylcholine juice which did not digest the di-peptide did not contain floccules.) The other two positive results came from duodenal and lower intestinal juices collected after cutting the post-ganglionic sympathetic nerves; these juices were opaque even after centrifuging and were the most turbid juices used in any experiment. In these four positive experiments from 20 p.c. to 80 p.c. of the substrate was split; maximum digestion took place at pH 7 once, at pH 8 twice and at pH 9 once, *i.e.* in agreement with the established optimum pH for "intestinal erepsin."

Poly-peptidase.—Eleven juices were tested, 9 from cat duodenum and 2 from cat jejunum or ileum. The four samples which digested the di-peptide digested peptone to a lesser extent (between 9 and 22 p.c. splitting of the substrate); and in addition another acetylcholine duodenal juice gave 14 p.c. digestion in spite of having been centrifuged.

cyanide is titrated directly with ceric sulphate in the presence of an oxidation-reduction indicator.

5. *Invertase.*—The tests were set up at the same time as those for amylase, and the same methods were used. The substrate was 5 p.c. cane-sugar and the experiments were set up at several H-ion concentrations between pH 5 and pH 9.

In all the above tests, digest mixtures consisted of equal quantities of juice, substrate solution and buffer. Incubation lasted usually for 16 hours, occasionally for 24 or 48 hours. Zero values for each experiment were obtained by estimating samples taken immediately after mixing juice and substrate. Blank experiments were carried out (1) with boiled juice and substrate, (2) with substrate and buffers, (3) with juice and buffers, incubated for the same length of time.

6. *Lipase.*—The juice was brought to pH 7 (blue-green to brom-thymol-blue) with 0·1 N HCl. 1 c.c. of neutralised juice (or 0·5 c.c. if more was not available) and 0·25 c.c. of triolein were mixed with 0·75 c.c. of 0·5 p.c. Na taurocholate or with water. Blank experiments with juice and triolein separately were also set up. The bottles were shaken mechanically in the incubator for 6 or 16 hours, then the contents were poured into beakers and the bottles washed twice with 3 c.c. of absolute alcohol, the washings being added to the digests. Increase in acid was estimated by titrating with 0·01, 0·02 or 0·1 N NaOH, using brom-thymol-blue as indicator.

7. *Enterokinase.*—Pancreatic juice was obtained by cannulating the pancreatic duct of a cat, eserinising, and then injecting acetylcholine subcutaneously and secretin intravenously. The trypsin in such juice is inactive and will remain so for weeks or months in the ice-chest.

Equal quantities of pancreatic and intestinal juice were incubated together for half an hour or more, then 1 c.c. of the mixed juice was set up for digestion with 0·5 c.c. of 5 p.c. egg albumen and 0·5 c.c. of buffer, pH 8 or pH 9. The amino-nitrogen was determined immediately after mixing and again after 16 hours' incubation. Controls were set up with pancreatic and intestinal juice separately and with pancreatic juice treated with an efficient activator supplied by Dr. C. L. G. Pratt.

Tests for enterokinase were also made on some samples of juice by utilising the capacity of pancreatic juice to clot milk in the presence of active trypsin [Mellanby, 1912–13; Kunitz and Northrop, 1933]. The results obtained by the two methods were identical.

Estimations of Neutralising Power of Juices.

The estimations were made on fresh juice as soon as it had been collected. The juice was titrated with standard HCl against brom-thymol-blue or phenolphthalein.

Maximum digestion took place at pH 7 twice, pH 8 twice, and pH 9 once. Three out of the other 6 duodenal samples gave a trace of digestion (less than 10 p.c.), but as the corresponding tests on the di-peptide were negative a technical fault was suspected though it could not be proved; in these three tests maximum digestion took place at pH 7 each time.

Amylase was present in every one of the 10 juices tested, 7 from cat duodenum and 3 from cat jejunum or ileum. Usually 40 p.c. or more of the starch was digested. In 7 quantitative tests maximum digestion took place at pH 7 four times, at pH 6 and 7 once, equally at pH 5, 6, and 8 once and at pH 4 in the test on post-ganglionic sympathectomy juice from the lower ileum. Digestion took place over a wide range of H-ion concentration, and between pH 5 and pH 8 the differences in the amount of splitting were small.

Invertase.—The same two acetylcholine juices which digested the di-peptide digested cane-sugar (qualitative). Of six other cat juices, two duodenal samples gave a trace of digestion and two gave none, and one lower intestinal sample gave a trace of digestion and another was qualitatively positive. In the three quantitative positive tests maximum digestion took place at pH 7 twice and at pH 5 once.

Lipase.—The same two acetylcholine juices which digested the di-peptide slightly digested fat. Seven other tests, all on cat duodenal juice, were negative; one of these negatives came from uncentrifuged acetylcholine juice, but this was the exceptionally acellular juice which also failed to digest peptides.

Trypsin was activated by every one of 9 juices tested, showing that *enterokinase* was consistently present. The juices were obtained by 5 different methods; one came from rabbit duodenum, 5 from cat duodenum, and 3 from cat jejunum or ileum.

Enzyme Tests on Juices from Permanent Duodenal Fistulæ. (Tables II. and III.)

Denervated (transplanted) duodenal fistulæ were made in 3 pigs, 3 cats, 2 rabbits, and 1 goat, and juices were tested for enzymes before and after the pedicle was cut. Innervated fistulæ (in which the muscle of the duodenum remained in continuity with that of the stomach and the mesentery was left intact) were made in 4 pigs, 2 cats, and 2 dogs. The results of the tests were similar in all animals and in both kinds of fistulæ.

Proteases were tested for in 36 experiments and *poly-* and *di-peptidase* in 33; the results in every case were negative. *Amylase*, on the other hand, was present in every one of the 18 juices tested; pig, dog and rabbit juice split from 45 p.c. to 80 p.c. of the substrate in 9 quantitative tests and cat juice slightly less (20 p.c. to 50 p.c.). As in the acute experiments, maximum digestion occurred at pH 5, 6 or 7 and the

TABLE I.—DIGESTION OF SUBSTRATES BY JUICE FROM ACUTE PREPARATIONS.

Source of juice.	Method of obtaining juice.	Enzymes sought.							
		Protoinases.		Peptidases.		Carbohydrases.		Lipase.	Entero-kinase.
		Albumen.	Casein.	Peptone.	Glycyl-glycine.	Starch.	Cane-sugar.	Triolein.	Albumen.
Cat Duodenum	Vagal stimulation	none none (pH 7 only) *	. . . .	trace trace none	none none none	40% . .	none . .	none none*	present present
	Vagal stimulation and splanchnicotomy	. .	. .	. .	none	40% 15%	none		
	Splanchnicotomy	none	none	none	none	50%	trace	none	present
	Post-ganglionic sympathectomy	. .	. .	21%	38%				
	Eserine	none (pH 7 only) *	none (pH 7 only) *	trace *	none * none none	present* 40% . .	trace	none * none * none	present present
	Acetylcholine	. .	. .	trace * none 14%	60% * none none *	present . .	present . .	trace * none *	present
Cat Jejunum and ileum	Splanchnicotomy	. .	. .	. .	none				
	Post-ganglionic sympathectomy	. .	. .	12%	80%	70%			
	Eserine	none (pH 7 only) *	none (pH 7 only) *	. .	none	46%	present trace	. . . .	present present
	Acetylcholine	. .	. .	22% *	20% *	present*	present*	trace *	
Rabbit Duodenum	Loop tied off under nembutal. No stimulation	. .	. .	. .	none				

TABLE III.—DIGESTION OF SUBSTRATES BY JUICE FROM PERMANENT DUODENAL FISTULÆ: (2) DOGS, CATS, RABBITS, GOAT.

Type of fistula.	Animal.	State of pedicle (in transplanted fistulæ).	Enzymes sought.								
			Proteinases.			Peptidases.		Carbohydrases.		Lipase.	Entero-kinase.
			Albumen.	Gelatine.	Casein.	Peptone.	Glycyl-glycine.	Starch.	Cane-sugar.	Triolein.	Albumen.
Innervated	Dog 1	..	none	none	none	none	none	80%	13%	none	present
	Dog 2	..	none	none	none	none	none	75%	trace	none	present
Transplanted	Cat 1	not cut	..	..	..	..	..	..	..	..	present
		cut	..	..	..	..	none				
	Cat 2	cut	..	..	..	none	none	20%	none	none	present present present
	Cat 3	cut	none	..	none	none	none	38%	none	none	present present
Innervated	Cat 4 2–4 weeks after operation	..	none	..	..	none	none	present	..	..	present present
	6 months later	..	none	..	..	..	none	50%	none	none none	present
	Cat 5	..	none								
Transplanted	Rabbit 1	not cut	none none	..	..	..	..	..	..	..	present
	Rabbit 2	cut	none	..	..	none	none	70%	trace	trace	none none
Transplanted	Goat	not cut	none	none	none	none none	none none	trace ..	none trace	trace trace	

TABLE II.—DIGESTION OF SUBSTRATES BY JUICE FROM PERMANENT DUODENAL FISTULÆ: (1) PIGS.

Type of fistula.	No. of pig.	State of pedicle (in transplanted fistulæ).	Enzymes sought.								
			Proteinases.			Peptidases.		Carbohydrases.		Lipase.	Enterokinase.
			Albumen.	Gelatine.	Casein.	Peptone.	Glycyl-glycine.	Starch.	Cane-sugar.	Triolein.	Albumen.
Transplanted	1	not cut	none	none	none	none none	none none	present present present	none present	none	
		cut	none	none	..	..	..	..	..	..	none none
	2	not cut	..	..	..	..	..	65%	..	..	none
		cut	none	..	none none	none ..	none ..	70% ..	none ..	none none	present present
	3	cut	none	..	none	none	none	60%	none	none	present
Innervated	4	..	none	none	none	none	none	present	..	..	present present
	5	..	none	..	none	..	none	45%	none	trace trace	present
	6	..	none	..	none	none	none	80%	trace	trace	present
	7	..	none	..	none	none	none	55%	trace	none none	present present

For explanatory notes see Table I.

juice from the upper part gave a value of 0·2 c.c. of acid and the juice from the lower part had no neutralising value (three experiments, vagal stimulation). Ileal juice was examined three times. A sample collected after eserine stimulation gave a value of 0·15 c.c. of acid, and two successive samples from a cat after post-ganglionic sympathectomy had the unexpectedly high value of 0·60 c.c. of acid.

The results of the few estimations on duodenal fistula juices (Table IV.) agree with those of Florey and Harding [1934]. Cat fistula juice has a slightly greater neutralising power than the juice from acute cat preparations and also than fistula juice from the dog and the pig.

Lysozyme Tests. (Table V.)

Cat juice contained no lysozyme (4 samples) and dog juice produced no immediate lysis and only partial lysis after standing overnight (3 samples). Pig juice, on the other hand, produced complete lysis at titres from 1 : 256 to 1 : 8192 after incubation for one hour (6 samples). Florey and Harding [1933] found weak lysozyme in rabbit duodenal juice, but in the one sample tested in the present series none was present.

TABLE V.—LYSOZYME TITRES OF JUICES FROM PERMANENT DUODENAL FISTULÆ AGAINST M. LYSODEIKTICUS.

Animal and preparation.	Titre : complete lysis except where stated otherwise.	
	After 1 hour at 37° C.	Same after 12 hours at room temperature.
Pig 2—Transplanted fistula	1 : 8000 (highest dilution used)	
Pig 3— ,, ,,	1 : 256	1 : 512
Pig 6—Innervated fistula	1 : 512	
,, (another sample)	1 : 512	
Pig 7—Innervated fistula	1 : 8192 (highest dilution used)	
,, (another sample)	1 : 256	1 : 512
Dog 1—Innervated fistula	No lysis	Partial lysis at 1 : 128
,, (another sample)	,,	,, ,, 1 : 32
Dog 2—Innervated fistula	,,	,, ,, 1 : 128
Cat 1—Transplanted fistula	No lysis	
,, (another sample)	,,	
Cat 2—Transplanted fistula	,,	
Cat 6—Innervated fistula	,,	
Rabbit 2—Transplanted fistula	No lysis	

We are indebted to Mr. L. A. Epstein for doing these tests.

differences within this range were small. A trace of *invertase* was found in 7 juices out of 15, the greatest digestion being 13 p.c. splitting after incubation for 48 hours (dog 1). The H-ion concentration at which maximum digestion occurred ranged from pH 5 to pH 7, as in the tests for amylase. Slight *lipase* action, greater in the presence of sodium taurocholate, was found in 6 out of 18 juices.

Enterokinase was present in all juices except those of one rabbit and one pig and the first of three samples from another pig; no explanation can be offered for its absence from these juices. In the positive experiments there was no significant difference in tryptic activity between test and activated control. In the milk-clotting test there was no difference in clotting time between test and control. The positive results were obtained at various times from 1 week to 6 months after making the fistulæ.

Tests for Cathepsin.

Taylor *et al.* [1938] found that casein was digested, mainly to proteoses, by normal human gastric juice acting at pH 7·4. There has been no evidence of a similar action by duodenal juice in the present work. A few juices from permanent fistulæ were activated with H_2S, and those also gave no evidence of a proteolytic action at H-ion concentrations between pH 4 and pH 8.

TABLE IV.—NEUTRALISING POWER OF JUICES FROM PERMANENT DUODENAL FISTULÆ.

Animal and preparation.	Neutralising power of juice in c.c. 0·1 N HCl.
Pig 3—Transplanted fistula	0·23
Pig 7—Innervated fistula	0·31
Dog 1—Innervated fistula	0·18
,, (another sample)	0·32
Dog 2—Innervated fistula	0·34
,, (another sample)	0·22
Cat 1—Transplanted fistula	0·35
Cat 2— ,, ,,	0·40
Cat 3— ,, ,,	0·42

Estimations of Neutralising Power.

Duodenal juice obtained by a variety of stimuli from acute cat preparations neutralised 0·2 c.c. of 0·1 N HCl per c.c. of juice twelve times and 0·3 c.c. seven times. One sample had no neutralising value and one neutralised 0·1 c.c. of acid. When the upper (Brunner's gland area) and lower parts of the duodenum were cannulated separately, the

experiments in which Savitch obtained secretion, apparently from the whole intestine, in response to vagal stimulation, were done on decapitate cats.

In our experiments the vagus has had less influence on the rest of the small intestine than on the duodenum. Vagal stimulation produces a watery secretion from a small part of the duodenum just distal to the Brunner's gland area. In two experiments stimulation of the vagus after splanchnic section caused secretion from the jejunum and ileum. The failure of the jejunum and ileum to secrete in response to simple vagal stimulation does not seem to have been due to inadequate stimulation, as in all experiments good gastric and duodenal secretion occurred.

Though section of the splanchnic nerves "liberated" the duodenum only, section of all preganglionic nerves caused secretion from the whole of the small intestine. Certain experiments support the idea that there is a "centre" in the spinal cord from which inhibitory impulses pass to the secretory mechanism of the gut. If the influence of this centre is removed by section of all preganglionic fibres the ganglia at the root of the mesentery take over inhibitory powers, their extirpation being followed by secretion. It is possible that the inhibitory effect of the splanchnics is so powerful that, under the present experimental conditions, vagal stimulation is unable to overcome it except in the duodenum.

Generally, to mimic vagal action by pharmacological means it is necessary to eserinise an animal and then to give acetylcholine. Eserine alone, however, causes intestinal secretion, though no other evidence of cholinergic action (*e.g.* salivary or tracheal secretion) appears. The addition of acetylcholine causes intense spasm of the intestine and epithelial cells and even intact villi are shed, while at the same time salivary secretion occurs.

The general conclusion appears to be justified that the gut, probably in its rich nerve plexuses, makes a relatively large quantity of acetylcholine, which is prevented from acting by the sympathetic nerves. The inhibition can be overcome experimentally by the administration of eserine or by section of the appropriate sympathetic nerves.

Although the effects of nerve stimulation and section can be demonstrated with regularity, there is as yet no evidence as to how they are integrated with intestinal function. For example, Thiry fistulæ of the jejunum and ileum, which retain their sympathetic innervation, do not, according to most observers, secrete juice during digestion. The small amount of "periodic" secretion [Boldyreff, 1928] occurs during starvation, not after feeding. One would have expected that if there were a general lowering of sympathetic "tone" during digestion secretion would occur in the fistulæ. The relation of the effects of local irritation or stimulation of the mucosa to the extrinsic nerves is even less under-

Discussion.

The Control of Secretion.—In reviewing the experimental findings it is convenient to consider first the control of the secretion in the upper part of the duodenum, which contains Brunner's glands. An abundant secretion of alkaline mucoid juice is produced from this part in acute cat preparations by (1) stimulating the vagi, (2) cutting the splanchnic nerves in the thorax, (3) administering eserine either alone or with acetylcholine. A scantier secretion is produced by the intravenous injection of material containing secretin. The consistency of the juice obtained by any of these methods is indistinguishable from that of juice from permanent duodenal fistulæ, though the cellular content varies with different methods of stimulation. It has been confirmed that the secretion of permanent transplanted duodenal fistulæ in cats is controlled by a hormone, as after 24 hours' starvation they are dry but secrete well after feeding. Similar denervated fistulæ in pigs react in the same way. The clear-cut results of these experiments leave little doubt that the secretory activity of the first part of the duodenum is under both nervous and hormone control.

In accordance with the current view on the chemical transmission of parasympathetic stimulation it may be supposed that acetylcholine is liberated either in the enteric plexuses or in the neighbourhood of the secreting cells or both, and that either the acetylcholine may be formed by vagal activity or "liberated" by the removal of sympathetic inhibition, or the breakdown of acetylcholine formed in the gut plexuses in the absence of stimuli may be prevented by eserine. Experimentally, the secretory effects of vagal stimulation, sympathectomy and eserine injection are all inhibited by atropine, which is evidence that they may act through a common mechanism. It seems certain that the juice obtained from the duodenum after cutting the splanchnic nerves is not a transudate from the blood, as it contains the "soluble" mucin typical of Brunner's gland secretion. The inhibition of the "paralytic" secretion by atropine and anæsthetics also supports this view.

The relative importance of the extrinsic nerves and the hormone mechanism in producing duodenal juice is difficult to assess. In acute experiments much more secretion can be produced by stimulating the vagus than by injecting secretin. In permanent preparations, on the other hand, transplanted fistulæ and fistulæ with the extrinsic nerves intact both stop secreting during starvation and start secreting immediately feeding begins (cats and pigs); both secrete about the same amount of juice and the composition of the juice from the two kinds of fistulæ appears to be identical.

The profoundly depressing effect of anæsthetics on intestinal secretion is probably one of the reasons why the control of the duodenal secretion has not previously been analysed. It is noteworthy that the

experiments in which Savitch obtained secretion, apparently from the whole intestine, in response to vagal stimulation, were done on decapitate cats.

In our experiments the vagus has had less influence on the rest of the small intestine than on the duodenum. Vagal stimulation produces a watery secretion from a small part of the duodenum just distal to the Brunner's gland area. In two experiments stimulation of the vagus after splanchnic section caused secretion from the jejunum and ileum. The failure of the jejunum and ileum to secrete in response to simple vagal stimulation does not seem to have been due to inadequate stimulation, as in all experiments good gastric and duodenal secretion occurred.

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stood, but there is good evidence that the jejunum and ileum deprived of their extrinsic nerves *can* produce juice when stimulated by a catheter [Ivy *et al.*, 1927; Nassett *et al.*, 1935].

Enzymes.—The enzyme content of duodenal juices produced in a variety of ways and from different species of animals has been investigated. In the introduction to Part II. various authors are mentioned who found protease, amylase, invertase, lipase and enterokinase in juice from duodenal fistulæ. The available writings of the Russian workers give no indication of the cellular content of their juices and possibly they, like many of the workers with jejunal and ileal fistulæ, were actually testing a mixture of pure secretion and cells. We have attempted, by eliminating cellular and bacterial enzymes as far as possible, to estimate the digestive properties of the actually secreted portion of duodenal juice.

Two groups of enzymes appear to be absent from cell-free juice—the proteases and the peptidases. No protease was found in any juice, and no peptidase in any juice from a fistula. Certain juices from acute preparations contained poly- and di-peptidase, but, with the exception of traces of poly-peptidase in three samples, there was a clear connection between peptide digestion and the high cell-content of the juices. Many samples of juice gave little or no deposit on centrifuging, so that nothing was removed to which enzymes might have been adsorbed, as Kestner [1930] and Pierce *et al.* [1935] argued.

Two other enzymes, invertase and lipase, which were present in cellular juices, sometimes appeared also in traces in other juices, but the splitting they produced was insignificant.

Two enzymes, amylase and enterokinase, were present in greater amounts. Amylase was present in every sample tested and usually split from 40 p.c. to 80 p.c. of the substrate. Waldschmidt-Leitz [1929] quoted work showing that starch was only digested to about 75 p.c. of the theoretical maximum splitting unless the products of digestion were removed; in 24 hours, therefore, many samples of juice produced something approaching maximum digestion. In his experiments on fistulæ from lower levels of the small intestine, Cajori [1933] found that amylase was the only enzyme in the juice whose digestive action was strong enough to keep pace with the absorption of the substrate; he suggested that it might come from the same source as the amylase of the blood and other body fluids.

In addition to amylase, an enterokinase capable of fully activating trypsin was found in nearly every sample of juice. Since its discovery by Schepowalnikow [Falloise, 1904; Pavlov, 1910; Babkin, 1928] there has never been any doubt of its presence in jejunal and ileal juice, but the only author to mention its presence in duodenal juice was Pavlov, who believed that the function of activating trypsin belonged more to the duodenal juice than to juice from lower levels of the small intestine.

Acute experiments gave unsatisfactory material for comparative tests. The comparison which we had hoped to make between the secretion of the duodenum and that of other levels of the small intestine proved hard to make, as the jejunum and ileum do not secrete in response to vagal stimulation, and other stimuli produce juice which is often very cellular. The comparison between the duodenal secretion produced by different stimuli in one species was also difficult, as the quantity and cellular content of the juice varies with the stimulus. Provided, however, that juice free from cells was obtained, no differences were found between the different methods of stimulation, and the few satisfactory tests on jejunal and ileal juice showed that, as in the duodenal secretion, amylase and enterokinase are present and di-peptidase absent.

The juice from permanent fistulæ, on the other hand, was consistently free from gross cellular contamination, so that the comparison between the duodenal secretion of five different species of animals, and between the secretion of transplanted fistulæ and those with their nerve supply intact, was satisfactory. There is no difference in the digestive enzymes of any of these secretions, though there are species differences in lysozyme content and possibly in neutralising power. Nasset *et al.* [1935] found no qualitative difference in the physical and digestive properties of juice from transplanted intestinal fistulæ before and after cutting the pedicle and we, with duodenal fistulæ, have found none.

The results of these enzyme investigations, which have been done by standard chemical methods and with the necessary precautions, conflict with those of Ponomarew [1902] and of Florey and Harding [1934], since, within the limits of the present investigation, the only enzymes constantly present in duodenal juice are enterokinase and amylase. Florey and Harding [1935 *a*] discussed the evidence that the mucus secreted in the duodenum was a product of Brunner's glands. Possibly the whole of the secretion which flows spontaneously from a permanent duodenal fistula comes from Brunner's glands or possibly part is contributed by the crypts of Lieberkühn. The only important enzyme action of the secretion appears to be the activation of trypsin, and this it shares with the secretion from other levels of the small intestine: the specific mucin content of the juice, however, and its alkalinity make it an admirable medium for protecting the first part of the duodenum against the acid gastric juice.

The close relation between di-peptide digestion and cell content in duodenal juice, and in the few satisfactory experiments on jejunal and ileal juice, leads us to believe that the peptidases (and the same probably applies to invertase and lipase) are endo-enzymes, in the sense that they are not secreted into the intestinal lumen in solution. Linderstrøm-Lang [1939] reviews recent work and considers that peptidases are shown to be typical endo-enzymes and he asks for reconsideration of the

15.00.	Stimulation started. Good motor activity of stomach and duodenum.	
	Duodenum.	*Stomach.*
16.00.	4·4 c.c. slightly bile-stained sticky fluid. 1 c.c. neutralises 0·2 c.c. N/10 HCl.	20 c.c. clear mucoid gastric juice.
17.00.	5·3 c.c. clean clear juice.	22 c.c. ,, ,,
18.00.	6·0 c.c. ,, ,,	25 c.c. ,, ,,
	Stimulation stopped. 40 c.c. saline given *subcutem.*	
19.00.	No secretion: stimulation recommenced.	
20.30.	Secretion had collected but was spilled by movement of animal.	
21.30.	3·0 c.c. duodenal juice. Stimulation stopped. 0·6 mg. eserine *subcutem.*	
21.50.	0·6 mg. eserine *subcutem.*	
22.00.	Stimulation recommenced.	
23.00.	5·5 c.c. clear fluid. Cat died at 23.10.	

In three experiments a cannula was tied into the jejunum instead of the duodenum. There was no flow of juice on stimulation. After stimulation for 2 hours the duodenum was severed 6 centimetres from the pylorus and cannulated; further stimulation then gave a flow of juice from the duodenum. It was thus apparent that the secretion had been forming in the duodenum and was being absorbed in the jejunum. Another experiment also illustrated that complete re-absorption of previously secreted juice is possible. Duodenal juice (6·0 c.c.), produced by vagal stimulation, was returned to a jejunal loop after atropinisation of the animal. It was completely absorbed in 2 hours 20 minutes; possibly the absorption was quicker than this but the preparation was not examined sooner.

It was thought that the whole of the secretion might be coming from the Brunner's glands of the duodenum, so a glass cone was fixed in the duodenum 2 centimetres only from the pylorus and both ends of the duodenum cannulated in the appropriate way. Stimulation of the vagi gave a good flow of juice from that part containing Brunner's glands and a lesser but still definite secretion from the duodenum containing none. The details of one of three experiments illustrating these points were as follows:—

Cat 100. Weight 2·5 kg.

10.30. Ether anæsthesia. Electrodes applied to infracardiac vagi. Common bile duct, pancreatic duct and accessory pancreatic duct tied. Obturators and cannulæ inserted. Decerebration completed at 11.45.

14.30. Animal in fair condition. Stimulus started.

17.30. Stomach—30 c.c. of clear mucoid secretion, 1 c.c. equivalent to 1·5 c.c. N/10 HCl. Upper duodenum—6 c.c. of clear slimy alkaline secretion, 1 c.c. neutralises 0·2 c.c. N/10 HCl; no precipitation with N/10 HCl. Lower duodenum—2·4 c.c. of slightly turbid secretion, almost watery in consistence and just alkaline: no neutralising value; turbidity on adding N/10 HCl.

Path of Vagus Supply to the Duodenum.—The path of the vagus supply to the duodenum was investigated by setting up an animal as above with the exception that two strong ligatures were left untied around the pylorus. After the rate of secretion on stimulation had been determined over 2 hours the ligatures were tied tightly and the pylorus cut through between them, care being taken to avoid interference with the blood supply. Further stimulation gave no significant secretion from the duodenum. Cutting the mucosa and submucosa only did not interfere with secretion. It was thus clear that the vagal supply to the upper duodenum passed from the stomach in the muscular layer of the gut wall.

Effect of Eserine on Vagal Stimulation.—The rate of secretion of duodenal juice during vagal stimulation was increased markedly by two doses of eserine (0·6 mg. each, given at an interval of 20 minutes), but it will be noted later that eserine in these doses may excite of itself a secretion of juice from the duodenum, jejunum and ileum.

In view of the failure to obtain juice from the jejunum or ileum by vagal stimulation attempts were made to "sensitise" the small intestine to the influence of the vagus. Eserine (0·6 mg.) was given *subcutem* to a cat and then 2 hours were allowed to elapse to see whether the eserine by itself produced a secretion. If there was no secretion the vagi were then stimulated, but no positive effects were obtained.

Action of Atropine.—Atropine (1·0 c.c. of 1 p.c. atropine sulphate) immediately caused a cessation of vagal secretion which lasted, even though stimulation was continued, for at least three hours.

The Effects of Anæsthetics.—The effect of basal anæsthetics on the rate of secretion from the duodenum was investigated in decapitated cats. After the rate of secretion had been determined by a two-hour control period of stimulation, 150 mg. of sodium luminal per kilo of body weight was given intramuscularly. Though this was not sufficient to cause a disappearance of the anal reflex, it caused almost complete cessation of secretion. This lasted during 3–4 hours of vagal stimulation. Administration of eserine gave some return of the vagal secretory effect. Details of such an experiment are as follows:—

Cat 169. 4·3 kg.

11.00. Ether anæsthesia. Decapitation. Vagi placed on electrodes. Common bile duct, pancreatic duct and accessory pancreatic

15.00. Stimulation started. Good motor activity of stomach and duodenum.

	Duodenum.	*Stomach.*
16.00.	4·4 c.c. slightly bile-stained sticky fluid. 1 c.c. neutralises 0·2 c.c. N/10 HCl.	20 c.c. clear mucoid gastric juice.
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Cat 169. 4·3 kg.

11.00. Ether anæsthesia. Decapitation. Vagi placed on electrodes. Common bile duct, pancreatic duct and accessory pancreatic

duct tied. Obturator placed in pylorus. Stomach and duodenum cannulated. Completed at 12 noon.

15.00. Stimulation started.
16.00. Collection started.
17.00. Stomach 17·5 c.c., 1 c.c. equivalent to 1·5 c.c. N/10 HCl.
Duodenum 5 c.c.
17.15. 650 mg. sodium luminal intramuscularly.
17.40. Anal reflex still present. Collection started.
18.40. Stomach 4 c.c., 1 c.c. equivalent to 1·5 c.c. N/10 HCl.
Duodenum 0·2 c.c.
Given eserine gr. 1/100 *subcutem.*
19.00. Eserine gr. 1/100 *subcutem.*
20.00. Gastric juice 6 c.c.
Duodenal juice 1·5 c.c.

Nembutal gave similar effects as follows:—

Cat 171. 2·8 kg.

9.45. Ether anæsthesia. Vagi dissected and placed on electrodes. Accessory pancreatic duct, common bile duct and pancreatic duct tied. Obturator placed in pylorus. Stomach and duodenum cannulated. Decerebration 10.55.

12.25. Stimulation of vagi started.
13.05. Secretion from both cannulæ flowing freely.
Collection begun.
14.05. Stomach 18 c.c.
Duodenum 3·6 c.c.
Cat given 1 c.c. nembutal (0·07 gm.) intravenously.
14.30. Collection started.
15.30. Stomach 4 c.c.
Duodenum 0·3 c.c.
15.40. Eserine gr. 1/100 *subcutem.*
16.00. Eserine gr. 1/100 *subcutem.*
16.20. Collection started.
17.20. Stomach 6 c.c.
Duodenum 1·2 c.c.

Ether similarly acted as an inhibitor. When a decerebrate animal had reached a steady rate of secretion of 20 c.c. per hour from the stomach and 5 c.c. per hour from the duodenum as a result of vagal stimulation, ether was administered for 1 hour. During this time scarcely any secretion occurred (stomach 3 c.c., duodenum 0·3 c.c.) in spite of the continuation of stimulation. The animal was left for 2 hours to allow elimination of the ether and then the vagi were stimulated again. Though by this time the animal's condition was poor it gave 10 c.c. of gastric secretion and 2·5 c.c. of duodenal secretion in an hour.

Of the anæsthetics tested, chloralose had the least effect on duodenal secretion. The rate of secretion in a decapitate cat during vagal stimulation was 8·4 c.c. per hour. After chloralose was given (70 mg. per kilo body weight) the rate of secretion was reduced to 5·5 c.c. per hour.

Some Properties of the Secretion.—The secretion which was obtained from the duodenum by vagal stimulation was a slightly opalescent, slimy fluid; at first it was bile-stained, but after 2 or 3 hours' flow it became colourless or only faintly yellow. It was alkaline to litmus and on collection had a pH of 8·3–8·4. On adding excess HCl and titrating back with NaOH using phenolphthalein as an indicator, 1 c.c. gave a neutralising value equivalent to 0·25–0·3 c.c. N/10 HCl. The chloride content was 0·45 p.c. There was only a slight increase of haziness on addition of N/10 HCl or 2 p.c. acetic acid, but the bulk of the mucus could be precipitated by phosphotungstic though not by trichloracetic acid. On standing on the bench overnight the sliminess disappeared from the juice. Vagal juice, as will be seen later, is indistinguishable from juice collected from a duodenal fistula.

From this series of experiments it is clear that stimulation of the vagi by the method used always causes secretion from the duodenum but not from the rest of the small gut, even when this is "sensitised" by a dose of eserine. The secretion from the duodenum is principally from the portion just distal to the pylorus, in which Brunner's glands are present.

The possibility existed that the secretion of the lower portion of the small intestine was under the control of the nervi erigentes, stimulation of which causes secretion in the colon [Wright *et al.*, 1938]. In one experiment done to investigate this point nothing was obtained from the ileum though good colonic secretion occurred.

(2) *The Sympathetic.*

Claude Bernard [1859] mentioned that the removal of the mesenteric (solar) ganglia caused a production of fluid in the intestine accompanied by diarrhœa. This phenomenon was more fully investigated in the dog by Moreau [1868] who found that section of the nerves accompanying the mesenteric vessels caused the accumulation of fluid in the loop of small intestine so denervated. This experiment has been confirmed by a number of workers who, for the most part, consider that the properties of the fluid entitle it to be considered true *succus entericus* [Hanau, 1886; Mendel, 1896; Falloise, 1904; Molnár, 1909 (using a denervated Thiry fistula)]. Leubuscher and Tecklenburg [1894] and Tecklenburg [1894] also repeated the experiment but concluded that the fluid was a transudate, a view supported by Starling [1911] who,

however, admitted that no proof was available against the view that secretion is inhibited by the sympathetic nerves.

The most thorough investigation of the nervous mechanisms involved was carried out by a committee of the British Association [Pye-Smith *et al.*, 1874; Brunton and Pye-Smith, 1875, 1876]. They also gave a résumé of the earliest work on this subject. They concluded that: "The secretory nerves of the intestines have the small ganglia of the solar and superior mesenteric plexuses for their centres; hence secretion is unaffected by section of the splanchnics, the vagi, or the dorso-lumbar part of the cord."

Mitsuda [1924], as the result of some not very convincing experiments, thought that the extra-intestinal parasympathetic is the secretory nerve for the intestine and that the extra-intestinal sympathetic is principally inhibitory to the glands by means of intra-mural sympathetic ganglia.

In the following section we record experiments dealing with this "inhibitory" influence of the sympathetics on secretion.

Effects of Cutting Greater Splanchnic Nerve.—For these experiments the chest was opened in the tenth interspace and the tenth rib divided near the transverse processes of the vertebrae. One pair of intercostal arteries were doubly ligated near the aorta and cut. Dissection just above the diaphragm then exposed a sufficient length of the splanchnic nerves to allow them to be cut and, if desired, drawn on to protected electrodes. The gut was prepared in the same way as for vagal stimulation. The animal was then decerebrated and left for the effect of the anæsthetic to wear off. It was found that after a variable period of from 1 to 5 hours the duodenum began to expel juice from the cannula. The highest rate of secretion from the duodenum was 6 c.c. per hour. There was no secretion from the jejunum, but occasionally the animal showed evidence of increased intestinal motility by passing fæces from the rectum.

The variable delay appeared to be due to the anæsthetic, for if the intestines of the animal were set up as described and then the cat was decerebrated and left for 2 hours before section of the sympathetic nerves (both can be rapidly divided *via* a short incision in the eleventh right intercostal space), the flow of secretion from the duodenum began at once. In some cases before the thoracic incision could be sewn up fluid was escaping from the duodenal cannula. This possible effect of anæsthetics was commented on by Mendel and Falloise.

This activity of the duodenum was not dependent on the continuity of the vagus nerves with the medulla, for if the vagi were divided below the lung root at the time of the initial operation division some hours later of the splanchnic nerves still gave rise to a rapid onset of secretion from the duodenum. Division of the vagi during "paralytic" secretion

had also no effect. The following protocol illustrates some of these points:—

Cat 144. 3 kg.

11.00. Ether anæsthesia, chest opened, splanchnics divided, chest closed. Common bile duct, pancreatic duct and accessory pancreatic duct tied. Obturator placed in pylorus. Duodenum 6 cm. from pylorus and stomach cannulated. Decerebration 12 noon.

17.00. 6 c.c. of secretion from stomach.
22 c.c. of sticky secretion from duodenum, 5·3 c.c. in the last hour.
Chest reopened and vagi divided without anæsthetic; animal did not react to handling of vagi. Completed at 17.10.

18.10. 5 c.c. of sticky duodenal secretion.
19.00. 3 c.c. of sticky duodenal secretion.
20.00. 3 c.c. of sticky duodenal secretion.

The seat of the inhibitory effect is below the third thoracic segment of the cord, for there was no secretion during 2 hours after cutting the neural axis of a decerebrate cat at the atlanto-occipital membrane and at the level of the third cervical vertebra, but division of the splanchnic nerves at the end of that period gave rise to secretion immediately.

The Effect of Stimulating the Splanchnic Nerves on the "Paralytic" Secretion.—When the rate of this secretion had become constant the splanchnic nerves were stimulated in the same way as the vagus nerves, a record of blood-pressure being taken from the femoral artery. Stimulation caused a rise of blood-pressure lasting slightly longer than the period of stimulation (due probably to the liberation of adrenalin from the suprarenal glands). The blood-pressure in the intervals between stimulation was somewhat above the initial resting level. As long as the stimulation was continued the rate of the "denervation" secretion by the duodenum was considerably diminished in all experiments. For example:

Cat 137. Male, 2·9 kg.

10.45. Ether anæsthesia. Chest opened and splanchnics cut and placed on electrodes. Common bile duct, accessory pancreatic and pancreatic ducts tied. Obturator placed in duodenum 6 cm. from pylorus and retrograde cannula passed through pylorus to drain duodenum. Stomach cannulated. Small gut cannulated 15 cm. below obturator in duodenum. Decerebration completed 12 noon.

14.00. Some secretion.

16.00. 14 c.c. of watery gastric juice.
9 c.c. of duodenal juice.
5 c.c. of jejunal juice.
17.20. 6 c.c. of gastric juice.
6·5 c.c. of duodenal juice.
3 c.c. of jejunal juice.
Stimulus started.
19.00. 2 c.c. of watery gastric juice.
1·5 c.c. of duodenal juice.
Nothing from jejunum.
Stimulus stopped.
20.20. 2 c.c. of gastric juice.
7 c.c. of duodenal juice.
1 c.c. from jejunum.

An attempt was made to investigate the nature of this inhibition by abolishing the vasomotor effect by ergotoxine.[1] As soon as the rate of secretion had become constant the animal was given ergotoxine (about 2 mg. of ergotoxine ethanesulphonate in divided doses) which abolished the response of the blood-vessels to adrenalin or sympathetic stimulation. Even when the blood-pressure remained at a high level following the ergotoxine secretion stopped. This made it impossible to elucidate the question further by this means.

Effect of an Anæsthetic.—Sodium luminal (150 mg. per kilo intramuscularly) reduced the rate of the "paralytic" secretion almost to zero in a decapitate cat, though the anal reflex was not abolished. Eserine (2 doses of 0·6 mg. at 20 minutes' interval) improved the rate of secretion under this narcosis.

Atropine.—Atropine (1 c.c. of 1 p.c. atropine sulphate intravenously) caused a cessation of this "paralytic" secretion for at least 3 hours.

Character of Fluid.—The fluid secreted had all the physical characters of that obtained by vagus stimulation, was chemically identical (so far as tested) and gave similar results to enzyme tests (see later).

The Relation of the Splanchnics to the Vagus.—It was thought that with the splanchnics cut, the small intestine other than the duodenum might respond to stimulation of the vagus.

Animals were prepared with the vagus nerves on electrodes, the intestines were cannulated as described and the animals were then decerebrated. Two hours later the chest was reopened without anæsthesia and the splanchnic nerves were divided. Two hours were allowed for the rate of "paralytic" secretion to be determined. In two experiments the rate of secretion from the duodenum was greatly

[1] We are indebted to Professor J. H. Burn for assistance and advice in conducting these experiments.

accelerated when the vagus was stimulated, while in one a meagre and in the other a copious flow of juice began from the small intestine. This intestinal secretion ceased when stimulation of the vagus ceased. The procedure of opening the chest a second time, and especially the handling of the splanchnics without anæsthesia, was severe and only these two animals lived long enough to give a satisfactory demonstration of the effect. For example:

Cat 152. Female, 2·8 kg.

10.45. Ether anæsthesia. Chest opened. Vagi placed on electrodes. Chest closed. Common bile duct, accessory pancreatic duct and pancreatic duct tied and cannulæ placed. Decerebration 11.45.

14.50. Chest reopened and sympathetics divided. 5 c.c. gastric juice had collected. Duodenal juice appeared at once.

16.50. 5 c.c. gastric juice.
3 c.c. duodenal juice.
No jejunal juice.
Stimulation of vagi begun.

18.10. 35 c.c. gastric juice.
12 c.c. duodenal juice, almost watery at the end of the period.
14 c.c. turbid, yellowish jejunal juice.
Stimulus stopped.

20.00. Stomach, 5 c.c.
Duodenum, 2 c.c.
No further secretion from jejunum.

From the foregoing experiments it is clear that the greater splanchnic nerve carries inhibitory fibres to the duodenum and possibly to the upper jejunum. The inhibitory centre is probably in the spinal cord. The integrity of the vagi is immaterial to the secretion of "paralytic" juice. "Paralytic" juice from the duodenum is identical in appearance with that produced by vagal stimulation, and as it contains Brunner's gland mucin cannot be looked on as a simple transudate.

As the greater splanchnic only carries inhibitory fibres to a small section of the upper part of the intestine, the possibility of the passage of inhibitory fibres to the cœliac ganglia by other nerves was investigated.

In the first place the experiment of Moreau was repeated, *i.e.* the effect was observed of the removal of the nerves accompanying the intestinal vessels to a loop of small intestine.

In most of such experiments a clear-cut confirmation of Moreau's results was obtained, *e.g.*:

Cat 212.

9.15. Decapitated.
12.45. Two loops of lower ileum were tied off and one denervated.
17.00. Abdomen reopened. Denervated loop was full of secretion. Innervated loop was empty.
18.30. Animal killed.

From the denervated loop, which was about 7 cm. long, 9 c.c. of fluid were collected; the fluid was turbid and contained much mucus. The control loop was moist, with little secretion; there were many tape worms in it.

Occasionally, however, secretion collected in loops whose innervation had not been interfered with (this happened three times in seventeen experiments). The reason for this is not clear.

To investigate the matter further, preparations were made in which all the preganglionic fibres to the cœliac ganglia were cut. The decapitate animals were prepared as follows:—

Through a midline abdominal incision the pancreatic ducts and bile duct were tied. A submucous ligature was passed and tied at the pyloric sphincter and cannulæ were inserted about 8 cm. down the duodenum and into the terminal ileum. In some experiments a cannula was also put in about half-way down the small intestine. The preganglionic fibres were then cut transperitoneally, in some animals after a control period of 2–3 hours, in others at the same time as the preparation of the intestines. It was found in general in this series of experiments that those animals secreted best which were denervated when the preparation was "fresh."

Good secretion was obtained in 6 cats. In 5, more juice was obtained from the jejunum and ileum than from the duodenum. In one animal 49·5 c.c. of duodenal juice and 19·1 c.c. of ileal juice were collected in 5½ hours. Details of an experiment are as follows:—

Cat 224.

11.00. Decapitated.
11.40. Pancreatic, accessory pancreatic and bile ducts tied.
Submucous ligature tied at pylorus.
All preganglionic nerves cut on both sides. Cannulæ tied into lower end of duodenum and terminal ileum. Abdominal window sewn in.
12.00. Operation finished.
14.15. 7·5 c.c. sticky duodenal juice.
7·5 c.c. sticky juice from ileum.
Since beginning of experiment very active peristalsis of small gut and cæcum. Small gut appears to contain a considerable amount of secretion.
15.20. 4·8 c.c. juice from duodenum.
18·0 c.c. juice from ileum.

16.10. 4·0 c.c. of duodenal juice.
Only a few drops of juice from ileum, but intestines look full.
Peristalsis still active.
17·40. 5·0 c.c. of duodenal juice.
1 c.c. of juice from ileum. Peristalsis now slight.
17.45. Killed. Intestines removed and emptied.
A total of 21·3 c.c. of juice was collected from the duodenum and 30 c.c. from the rest of the small intestine. The stomach contained several c.c. of thick mucoid acid secretion.

In one animal the greater and lesser splanchnics only were cut. This gave secretion from both the duodenal and ileal cannulæ, but the quantity was less than in those experiments in which all preganglionic fibres were cut.

In two control cats in which the abdominal contents were displaced as if for cutting the nerves and received considerable handling no juice was obtained from the small intestine.

Action of Ganglia at Root of Mesentery.—The possibility existed that the cells of the cœliac ganglia might themselves exert some inhibitory action, *via* the mesenteric nerves.

At aseptic operation all preganglionic fibres and a portion of the abdominal sympathetic chain were removed transperitoneally. For 2 to 5 days following this the cats passed loose motions, but thereafter behaved as normal animals. Some 10 to 14 days later they were decapitated, the intestines were prepared as before described and the ganglia surrounding the mesenteric artery were then removed. These cats gave quite remarkable responses. In the first cat 4·8 c.c. of duodenal and 28·3 c.c. of ileal juice were produced in 3½ hours, in the second 7·6 c.c. of duodenal and 48·4 of ileal juice in 4 hours, in the third 5·7 c.c. duodenal and 22·6 c.c. ileal juice in 2½ hours and in the fourth 3·0 c.c. duodenal and 20 c.c. ileal juice in 4 hours.

The following protocol shows that juice did not flow till the ganglia were removed:—

Cat 238*a*.

6th March 1939. Ether. Aseptic operation.
Midline abdominal incision. Gut, etc., pushed to one side and splanchnics and all visible branches going to cœliac ganglia cut. Portions of abdominal sympathetic chain with ganglia taken out. Abdomen sewn up with fine silk.
8th March. In good condition. No diarrhœa.
11th March. Took only milk, refused meat. Some diarrhœa.
16th March. In good condition, has been eating well, no diarrhœa. Has been starved for the last 24 hours.

12.00 noon. Decapitated. Duodenum and lower ileum cannulated. Main pancreatic and bile ducts tied. Submucous ligature tied at pylorus.
14.00. Few drops of juice present in both cannulæ. Ganglia removed.
15.20. Duodenum 1·8 c.c.
Rest of small gut 6·2 c.c.
16.30. Duodenum 3·2 c.c.
Small gut 4·8 c.c.
17.10. Small gut 16·3 c.c.
17.25. Small gut 8·8 c.c.
18.00. Duodenum 2·6 c.c.
Small gut 5·1 c.c.
11·0 c.c. clear juice in stomach.

The character of the intestinal juice varied considerably. In some cases it contained much thick mucus mixed with opaque white material. In others, especially the later portions to be collected, the juice was fluid, though usually somewhat turbid. The duodenal juice always preserved the physical characters already described, but it also was turbid.

From all the experiments on "paralytic" secretion, it can be said that cutting the greater splanchnic in the thorax "releases" the duodenum only and that cutting all preganglionic fibres "releases" the whole of the small intestine; if recovery is allowed after cutting the preganglionic fibres the cœliac ganglia take over an inhibitory rôle, as shown by the free secretion which occurs if they are later removed.

The Effects of Eserine and Acetylcholine.

As a preliminary to testing the effects of acetylcholine, eserine was given to decerebrate animals with the intestines cannulated as usual, but with the nerve supply intact. In some cats, instead of the usual two doses of 0·6 mg. of eserine *subcutem* at 20 minutes' interval, three or even four doses of this amount were given at the same interval. While waiting for the drug to take effect before giving acetylcholine we were surprised to see secretion appear from the cannulæ in the small intestine and duodenum. This observation was frequently confirmed. Quite large quantities of juice could be collected from both portions of the intestine. The amount of eserine necessary to produce this effect was somewhat variable; in some cats secretion began after only two doses of 0·6 mg., and in no cat were more than four doses necessary. Similarly, there was great variation from animal to animal in the frequency with which it was necessary to repeat the administration of eserine to keep the flow going. In no case was there a failure of secretion from the duodenum, but in some cases (two out of seven experiments) there was a failure of secretion from the small intestine other than the duodenum. No significant increase of secretion from the stomach

occurred. The eserine was effective whether or not the vagus nerve had been cut below the lung root. Atropine stopped its effect and sodium luminal (150 mg. per kilo) nearly did so. In no case in which eserine produced a flow of intestinal juice was there any secretion of sweat on the pads of the feet, of mucus in the trachea or of saliva.

The effect of eserine is shown in the following protocol:—

Cat 108. Female, 3·00 kg.

12.30. Ether anæsthesia.

Bile and accessory pancreatic ducts tied, pancreatic duct cannulated. Submucous ligature tied at pylorus, duodenum cannulated and a loop of small gut 15 cm. long tied off and cannulated.

14.20. 0·6 mg. eserine *subcutem*.

14.40. 0·6 mg. eserine *subcutem*.

15.00. 0·6 mg. eserine *subcutem*.

16.42. 0·6 mg. eserine *subcutem*.

17.00. From stomach 2·1 c.c. of juice.

From duodenum 6·5 c.c. of sticky juice, 1 c.c. neutralises 0·3 c.c. N/10 HCl; the mucus is not precipitated by adding acid.

From ileum 22·0 c.c. of slightly sticky juice, 1 c.c. neutralises 0·15 c.c. N/10 HCl; the mucus is precipitated by adding acid.

18.05. Drop or two from stomach.

4·3 c.c. duodenal juice.

0·8 c.c. ileal juice.

18.35. 0·6 mg. eserine.

19.05. 4·1 c.c. duodenal juice.

12·0 c.c. ileal juice from intestinal loop (from which tip of cannula had come out).

The effect of eserine was also tested on a cat bearing a duodenal fistula. A dose of 0·6 mg. was given *subcutem* to this animal after it had been starved to abolish secretion from the fistula. The animal rapidly showed signs of excitement, inco-ordination and itching. Secretion soon appeared at the mouth of the fistula and continued for an hour. The excitement of the animal prevented collection of the secretion.

The secretion produced in the duodenum by eserine was sticky and almost clear and had the characteristics described for the juice produced by vagal stimulation. That from the small gut differed in being watery with no stickiness whatever, and even the last portion of as much as 35 c.c. of secretion collected from a small loop was turbid from cells floating in it. It was just alkaline to litmus and had no significant neutralising value.

Acetylcholine was given *subcutem*, after eserinisation, to decerebrate

cats of which the intestines had been cannulated. The maximum safe dose for animals which had been prepared as described and had been left for 2 hours to avoid anæsthetic effects was 2 mg. This dose gave a definite increase in the rate of secretion from the small intestine, or started secretion if not already present, and gave a slight increase in gastric secretion. When, however, a full rate of secretion had been excited by four doses of 0·6 mg. eserine, acetylcholine did not increase the rate of secretion. The intestinal juice changed somewhat in character after giving acetylcholine. The change ranged from an increase in the turbidity of the juice, through an intermediate stage with "clots" of cast-off villous epithelium, to a juice in which villi and some blood were present. At the post-mortem examination the intestines which had shed villi showed localised hæmorrhagic patches in the mucosa up to 1 cm. in diameter from which the villi both macroscopically and microscopically were gone. This effect was inhibited by atropine sulphate (1 c.c. of a 1 per cent. solution given intravenously).

Influence of Hormones on Intestinal Secretion.

Delezenne and Frouin [1904] were the first to report an abundant secretion from a fistula in response to the intravenous injection of secretin-containing preparations. Their fistula was made from the duodenum (below the pancreatic duct) and upper jejunum of a dog. Bottazzi and Gabrieli [1905–06], in acute experiments on dogs, found that large injections (15–40 c.c.) of duodenal extracts produced a secretion of clear and slightly yellow juice which sometimes contained mucus and debris; they thought the active substance was a "secretin" similar to pancreatic secretin. Foà [1908] could not always obtain a secretion by Bottazzi and Gabrieli's method, and many dogs were killed by the injections. Brynk [1911] failed to confirm Delezenne and Frouin when he put 200–300 c.c. of HCl of different strengths into the stomach and got no increased secretion from a Thiry-Vella fistula. Delezenne and Frouin had obtained a secretion by this procedure, though they were clear that the stimulus came from the intestine, not from the stomach. Frouin [1905] found that the intravenous injection of *succus entericus* itself caused intestinal secretion; his result is possibly explained by the observation of Volborth [1925] that the deposit from intestinal juice contains a "secretin" which stimulates the pancreas (though Frouin noticed no effect on the pancreas from his injections). Ågren [1934] reported that injections of highly purified secretin products caused intestinal secretion, though his results are perhaps not very clear cut. Authors have also reported stimulation of intestinal secretion by a variety of apparently non-specific substances [Mironescu, 1909; Hirata, 1910; Komarov, 1924 *a* and *b*, 1926]. Ivy *et al.* [1927] noted incidentally that loops of intestine transplanted beneath the skin

secreted "normal" intestinal juice, presumably in response to mechanical stimulation. Florey and Harding [1935 *a* and *b*] transplanted the first part of the duodenum subcutaneously in cats and showed that such denervated fistulæ responded to feeding after starvation by secreting a clear mucoid juice; no local or artificial stimulation of any sort was used. The same fistulæ secreted in response to an intravenous injection of highly purified secretin. Nasset *et al.* [1935], Nasset [1938], and Nasset *et al.* [1938] performed experiments demonstrating the possibility of a humoral stimulus to intestinal secretion, but they doubted whether secretin was the hormone concerned. They described a new hormone, enterocrinin, which they believed was the substance involved.

The following experiments on the influence of hormones on secretion were performed exclusively on that part of the intestine containing Brunner's glands.

Cats.—We have repeated the transplantation of the duodenum on four cats with results which agree with those previously reported. There can be no reasonable doubt that some humoral mechanism exists in the cat for the activation of the upper part of the duodenum. Intravenous secretin caused secretion from these fistulæ.

Pigs.—The humoral control of duodenal secretion has also been investigated in the pig. Transplantation methods were again used. A portion of duodenum from between the bile and pancreatic ducts was isolated [Goodfriend *et al.*, 1938] and placed subcutaneously while still retaining its mesenteric pedicle, in the way already described for the cat and rabbit [Florey and Harding, 1935 *a*]. Six weeks later the pedicle was cut, thus isolating the fistula beneath the skin of the abdominal wall.

Three pigs were operated on in this way, and in all the fistulæ have secreted in response to food, even on the day following the cutting of the pedicle. The following protocol shows the amounts of secretion obtained in one of these pigs with a subcutaneous fistula:—

The pig was starved for 24 hours, but was allowed water. The secretion was then collected.

1st hour, 0·8 c.c.
2nd hour, 0·1 c.c.
3rd hour, 0·0 c.c.
4th hour, 0·0 c.c.

The pig was then fed.

1st hour after feeding, 9·8 c.c.
2nd hour after feeding, 13·1 c.c.
3rd hour after feeding, 13·0 c.c.

The secretion was typical mucoid duodenal juice.

Thus in the pig as well as the cat the duodenum secretes a mucoid fluid in response to food after all extrinsic nervous influences have been removed.

Fully innervated fistulæ of the pig's duodenum have been made for comparison with the foregoing isolated fistulæ. As this type of fistula has not been prepared before in the pig a description of the method is given.

A young pig (usually weighing about 12 kg.) was starved for 24 hours before operation. Open ether anæsthesia was used. The pyloric end of the stomach was brought out through a midline incision. A cut was made through the muscle, parallel to the long axis of the pylorus, and the mucosa was dissected off the muscle all round the pylorus with as little disturbance to the muscle as possible. The mucosa was then cut through, the upper cut end was insewn and the lower part was excised as far as the beginning of duodenal mucosa. The bared gastric muscle was brought together by a few loose stitches and the longitudinal cut in the muscle was sewn up. In this way the lumen of the duodenum was separated from that of the stomach, while leaving the nervous pathways in the muscle and along the vessels intact. Next the bile duct was detached from the duodenum; in some cases a piece of duodenal mucosa round the entrance of the duct was excised, in others the duct was cut across where it entered the intestinal muscle. The hole left in the duodenum was sewn up. The duodenum was cut across above the pancreatic duct. The lower cut end was insewn and the bile duct was anastomosed to this part of the duodenum near the suture-line. If the papilla had been excised with the duct the mucosa surrounding it was sewn into a small hole in the duodenum. If the duct had been cut across the best procedure was found to be to tie a glass cannula into the free end of the duct; a short piece of rubber tubing was slipped on to the cannula to make subsequent manipulations easier. A small hole was made in the duodenum through which the rubber tube was pushed, followed by the glass cannula and the cut end of the duct. A purse string suture previously placed around the hole in the gut was tied round the duct and cannula and, to hold it more firmly, the duct was sutured into an appropriately placed gutter made by infolding the walls of the duodenum. We consider this procedure easier than transplanting the papilla of the bile duct. A gastro-enterostomy was made to a convenient loop of intestine. The upper cut end of the duodenum was brought to the midline abdominal incision to form the opening of the innervated duodenal fistula. Animals so operated have been kept in health for months after operation. Three were killed for reasons unconnected with the fistula at 2, 6, and 7 months after operation; one is in good condition at the time of writing, 8½ months after operation.

Such a fistula secretes considerable quantities of mucoid juice, exactly similar in physical appearance to that secreted by a totally denervated fistula. These fistulæ also react to starvation and feeding in the same way as the denervated fistulæ; for example:—

The pig was starved for 24 hours, but allowed water. The secretion was then collected.

1st hour, 1·2 c.c.
2nd hour, 0·3 c.c.
3rd hour, 0·0 c.c.
4th hour, 0·0 c.c.

The pig was then fed.

1st hour after feeding, 15·8 c.c.
2nd hour after feeding, 17·3 c.c.
4th hour after feeding, 17·0 c.c.

Dogs.—Some observations on the effects of starvation and of secretin injections were also made on upper duodenal fistulæ in dogs. Ponomarew [Babkin, 1928] worked with dogs and appears to have been the first to prepare fistulæ of that part of the intestine containing Brunner's glands. His preparations (2 dogs) gave a small flow of mucoid alkaline juice which appeared to be uninfluenced by starvation. Ponomarew's fistulæ were presumably innervated by the vagus, as he closed the pylorus by a submucous ligature leaving the muscle intact. Florey and Harding [1934] made fistulæ in 2 dogs in which the stomach was cut right across at the pylorus; these fistulæ were therefore partially denervated. An alkaline mucoid juice was obtained—about 1·5 c.c. per hour—the flow of which was reduced but not abolished by 24 hours' starvation.

In the present work difficulties were encountered in preparing the fistula in one operation. Although the surgical procedures appeared to be adequate the first three dogs died 3, 5, and 11 days after operation. There was nothing obvious to account for these deaths as the dogs were not greatly shocked after operation and appeared to do well until they died quite suddenly. By adopting the following two-stage procedure we prepared two very satisfactory animals.

At the first operation the mucosa of the stomach just proximal to the pylorus was separated from the muscle by blunt dissection through an incision placed longitudinally in the gastric muscle near the greater curvature. In this way a minimum of disturbance to the nerves passing from the stomach to the duodenum was ensured. The mucosa was then cut across and the proximal end insewn; the distal end was removed as far as the pyloric ring, which was then closed by sutures. The incision in the gastric muscle was sewn up and a gastro-enterostomy was made to the first part of the jejunum. The abdomen was sewn up and the animal allowed to recover for 14 days.

At the second operation the duodenum was cut across just above the bile duct and the distal end was insewn, avoiding compression of the bile duct. The proximal cut end was brought to the surface in the upper part of the midline abdominal incision and sewn to the muscle

and skin. The animals were fed on milk, syrup and crushed biscuit, with meat after the first 3 days. They are alive and well 2½ and 3 months respectively after the second operation. The fistulæ secrete a clear mucoid juice which has floating in it small white flecks of débris some of which are difficult to centrifuge out. The juice is either colourless or faintly brown. The following are some figures illustrating the effects of 24 hours' starvation and subsequent feeding:—

After 24 hours' starvation (water allowed) a pot was tied over the opening of the fistula.

1st hour, 0·0 c.c.
2nd hour, 0·0 c.c., fistula completely dry.
3rd hour, 0·0 c.c., fistula completely dry.

The dog was then fed.

1st hour after feeding, 0·9 c.c.
2nd hour after feeding, 1·3 c.c.
3rd hour after feeding, 1·5 c.c.

The other dog gave a similar result and secreted 1·6, 2·1, 2·0 and 2·0 c.c. of juice in the first 4 hours after feeding. The fistulæ in these dogs therefore reacted to starvation in the same way as those in cats and pigs, and gave decisive evidence that a flow of juice is produced by feeding in this species.

Injections of crude secretin were also given:

Dog 1. The dog was starved as before. In the two hours before the injection no juice could be collected. 2 mg. of secretin were injected intravenously and in the next half-hour 0·8 c.c. of typical sticky duodenal juice was collected. This rate was approximately the same as that after giving food.

Dog 2 was also starved. No juice could be collected before the injection and the mucosa looked dry. 2·5 mg. of secretin were injected and secretion was seen inside the fistula within 2 minutes. About 0·5 c.c. was collected in the 15 minutes after the injection, but as the pot slipped an exact measurement was not obtained.

Acute Experiments.—The evidence for a humoral control of the duodenal secretion received support from experiments done with decerebrate cats. To test the effect of secretin the intestine and pancreatic duct were cannulated in the usual way. A dose of secretin which gave 2 c.c. of pancreatic juice gave 0·3 to 0·5 c.c. of tenacious mucoid secretion from the duodenum. Similar secretion occurred each time the dose was repeated.

It was thought that the secretin might affect the Brunner's glands in the same way as the pancreas, exciting an outpouring of alkali as well as mucus. A free flow of eserine juice was excited before the secretin was given to animals prepared as usual. The alkalinity of the juice was compared before and after the secretin; no significant increase

occurred. When the secretin was given during stimulation of the vagus there was similarly no increase in alkilinity.[1]

Effects of Histamine.—Koskowski [1926] stated that subcutaneous histamine injections caused a secretion of intestinal juice from a Thiry-Vella fistula in dogs. He did not, however, give any figures of the quantities obtained. Nechoroschew [1929], using dogs with fistulæ and decerebrated cats and dogs, also concluded that histamine given enterally caused an increase in the motor and secretory activity of the intestine. From his description it is difficult to know exactly how his experiments were done and the amounts of intestinal secretion reported were very small. Cajori [1933] examined histamine juice produced from dogs reported on later by Berndt and Ravdin [1934]. These authors used a modified Thiry loop with Johnson's [1932–33] catheter. They noticed a considerable increase in the amount of secretion following the subcutaneous administration of histamine. The increase reached its maximum half an hour after the injection. On the contrary Lim *et al.* [1922–23] found no evidence of a secretory effect on the human duodenum. Florey and Harding [1934], from experiments on decerebrate cats, concluded that histamine did not stimulate the secretion of Brunner's glands.

In the decerebrate cat, in the present series of experiments, we were not able to collect any secretion from the duodenum after 3·5 mg. histamine phosphate, given subcutaneously in divided doses. The stomach secreted 85 c.c. of watery acid juice during an experiment which lasted 4 hours, but no fluid appeared in the duodenum. The administration of eserine then gave a copious flow of duodenal secretion, showing that the failure of secretion was not due to any fault in the preparation.

It is possible that some of the positive effects reported were due to the liberation of acid gastric juice which passed into the intestine to liberate a hormone acting on the intestinal mucosa (*cf.* section on hormone stimulation).

Histological Investigations.

Stimulation of the vagi causes great depletion of the mucin contained in Brunner's glands; in some cases almost complete exhaustion occurs (figs. 1 and 2).

The effect on the crypts of Lieberkühn was in some experiments negligible, but in others there was flattening of the epithelial cells with apparent dilatation of the lumina of the crypts, in which there was coagulated material.

Brunner's glands also showed evidence of exhaustion following section of the splanchnics, and in some specimens there was œdema of

[1] The late Professor J. Mellanby very kindly supplied the secretin used in these experiments.

the tips of the villi. Eserine also produced histological evidence of activity of Brunner's glands in some cases, but when acetylcholine was also administered great exhaustion of these structures was apparent. In nearly all experiments using acetylcholine, however, there was

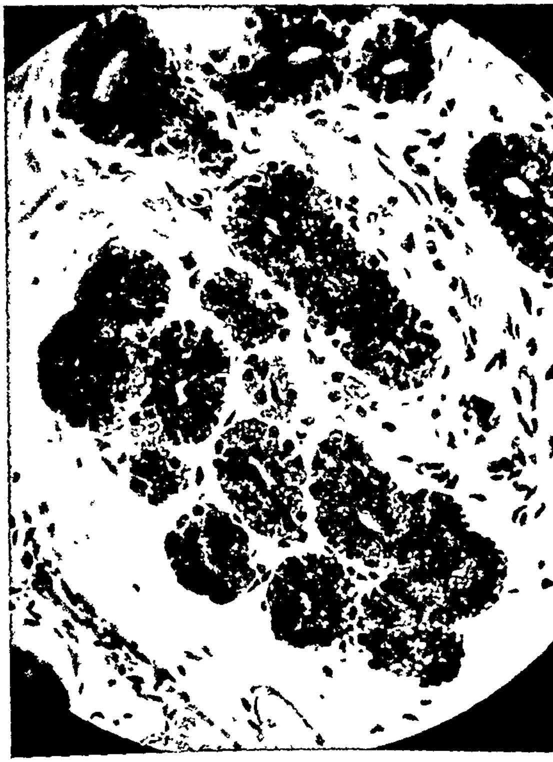

FIG. 1.—Alveoli of Brunner's glands from a cat starved during the previous 24 hours. Mucin (stained with gentian violet) can be seen to fill the alveolar cells, the nuclei of which are flattened towards the cell-base.

damage to the mucosa varying from slight hæmorrhages in the villi to desquamation and destruction of the superficial mucosa.

In the Moreau-type experiment the appearances were variable. In some there was considerable destruction of the surface epithelium, while in others, even if the secretion had been good, no such thing occurred. In some cases there was flattening of the epithelial cells of the crypts, while the villi retained their normal appearance.

To sum up, such histological observations as have been made fully bear out the experimental data concerning the activity of Brunner's glands, but the present collection of material does not give any clear-cut or consistent histological picture of the effects of the various procedures on the other intestinal structures.

Fig 2 —Alveoli of Brunner's glands exhausted of their mucin following vagal stimulation. Note wide lumina of alveoli, traces of darkly stained mucin at the free borders of the alveolar cells and the rounded clearly seen nuclei each with its nucleolus.

Part II. Enzyme Investigations.

Introduction.

Duodenal Juice.—Little work has been done on the digestive function of juice from the Brunner's gland area, because in all species it is a small part of the intestine and relatively difficult of access. Colin [1854] collected duodenal juice from the horse and accurately described its physical characters. Kuhne [1868] stated that the Brunner's gland area gave a viscous extract which did not digest fat, and Brown and Heron [1879–80] found that this distinctive extract had the most feeble digestive power of any extract from the small intestine. The St. Petersburg school (working entirely, so far as is known, with dogs) reported that duodenal juice contained pepsin and rennin-like enzymes [Ponomarew, 1902, quoted by Babkin, 1928; Pawlow and Parastschuk,

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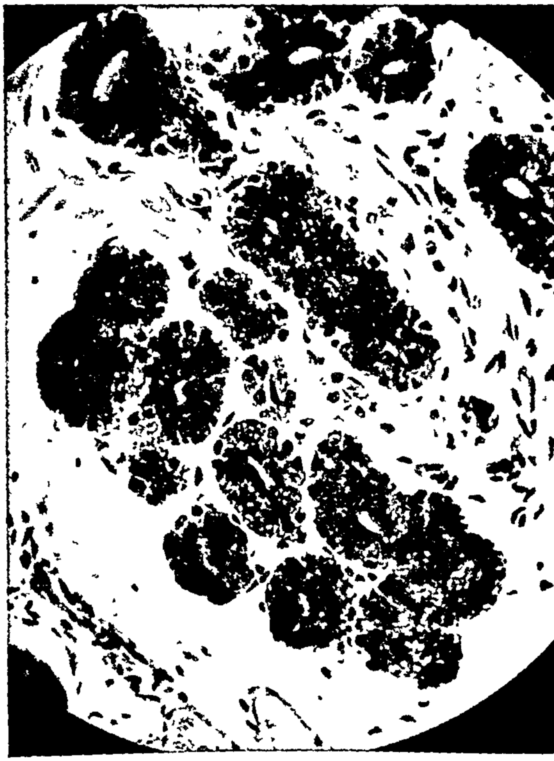

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from ruptured cells, and a stronger amylase and [1935] traces of lactase.

Babkin [1928], in his review of intestinal secretion, stated that feeble amylase and lipase, stronger invertase, maltase and lactase and also nuclease, erepsin and enterokinase were present in the juice.

Sources of Reported Enzymes.

Intestinal juice consists of a secreted fluid together with intestinal bacteria and cast-off cells, so that beside the secretion possible sources of enzymes are bacteria and ruptured cells. Traces of gastric and pancreatic enzymes adhering to the mucosa could be found in acute or recent preparations.

Leube [1868] found that filtered juice did not always digest cane-sugar, whereas the whole juice did so, and he was the first to suggest that the enzymes originated in the "Flocken" and passed from them into the fluid part of the juice.

The "Flocken" of the German literature were floccules of mucus mixed with opaque matter which sank in the juice; they were present in juice obtained by a variety of methods of stimulation. As an extreme instance, Masloff [1882] reported a mass occupying a third of the volume of the juice. Microscopic examination showed the floccules to contain "mucous cells" [Masloff], round cells [Dobroslawin, 1870], leucocytes [Gumilewski, 1886], "fat" from cell debris [Röhmann, 1887], and coccoid bacteria [Pregl, 1895]. The cells were usually very degenerate. Hermann and Ribère [1931] found leucocytes, desquamated epithelial cells and mucus in juice from a human fistula. Even in relatively clear juice a small deposit can usually be obtained on centrifuging and shown to consist of amorphous material containing bacteria and a few still recognisable cells [Florey and Harding, 1934]. Kühne [1868] was one of the few workers who described juice from a fistula as being clear and free from floccules.

Ramond [1904] observed that mucosal cells and leucocytes were shed from the mucosa of the intestine during digestion. In the fistula, peristalsis and the normal growth of the mucosa may similarly cause cells to be shed into the juice, while partial obstruction or slight sepsis at suture-lines would account for the presence of polymorphs and of non-intestinal bacteria. Leube found that solid matter accumulated in the fistula during starvation, when no fluid was being secreted.

(a) *Cell Enzymes.*—No one disputes that the epithelial cells of the intestinal mucosa contain enzymes which can split food substances, for example, peptidase (erepsin) [Cohnheim, 1901] and invertase [Paschutin, 1871; Bernard, 1873]. Less is known about the enzymes of round cells and polymorphs. A number of workers have compared turbid

1904] and a feeble or inconstant amylase, invertase, and lipase [Ponomarew]. Abderhalden and Rona [1906] confirmed the pepsin-like enzyme. Pavlov [1910] said that trypsin was chiefly activated by the duodenal secretion. In 1934 Florey and Harding, using rather crude methods, tested for enzymes in juice from duodenal fistulæ in the goat, pig, dog, cat and rabbit. They found a pepsin-like enzyme in the goat and dog, and a trace of lipase in the dog.

Juice from the Jejunum and Ileum.—In contrast with duodenal juice, the *succus entericus* from the rest of the small intestine has often been investigated. Frerichs in 1846 claimed to be the first to tie off an empty loop of small intestine and collect and describe the juice which accumulated in it. The same method was used by Colin [1854], but was superseded in 1864 by Thiry's operation for making a permanent fistula, subsequently modified by Vella [1888].

A great many experiments were reported in the fifty years after Thiry described his operation, culminating in a spate of work from the St. Petersburg school after 1899, summarised by Pavlov in *The Work of the Digestive Glands* [1910]. Chemical methods were primitive and possible fallacies often not recognised, so nothing is to be gained by reviewing the work of this period in detail; there were many negative results, but the enzymes reported present by one or another worker included amylase, invertase, maltase, lactase, arginase and trehalase, splitters of fibrin, egg albumen, meat and casein, a rennin, a lipase, a fat emulsifier, splitters of peptone and peptides (erepsin), and enterokinase.

Recent workers have also found a variety of enzymes. Orbéli and Sawitch [1917] found in human intestinal juice amylase, invertase and maltase, lipase, erepsin and enterokinase, but no lactase. In human ileal juice, Hermann and Ribère [1931] found no enzyme digesting egg-white, a doubtful lipase, a strong invertase, feeble amylase and enterokinase, and an erepsin which was strong during digestion and weak or absent in starvation. Bickel and Kanitz [1934] found amylase and erepsin, but no lipase, in mucus-free samples of uncentrifuged human juice.

In dog intestinal juice Roger and Binet [1921] found lipase, le Breton and Mocoroa [1931] found peptidase and enterokinase but no trypsin-like enzyme, and Koskowski [1926] found amylase and invertase, but described them as endo- (*i.e.* cell) enzymes. Schwichtenberg and Burget [1931–32], using juice from closed intestinal loops, found invertase in 75 per cent. of jejunal and 50 per cent. of ileal samples, and lipase occasionally. Pierce *et al.* [1935], who macerated the deposit with the supernatant juice, found amylase, invertase, maltase, lactase, erepsin and a feeble lipase; casein and albumen were not digested at pH 6, but gelatine was slightly digested when bile was present. Cajori [1933] found feeble erepsin and invertase, which he thought came

proteins themselves." They adduced no chemical or other evidence for this supposition, but supported it [Nasset *et al.*, 1935] by calculating that 0·5 mm. of mucosa would have to be shed from the surface of a Thiry fistula to produce the amount of solid material in a 6–8-hour collection of juice. Their method of collection, however—passing a catheter to the end of the fistula—may damage the mucosa and rub off many cells.

Nearly all other authors who have considered the point have concluded that some of the enzymes reported in intestinal juice are endo-enzymes from the mucosal cells and not exo-enzymes secreted into the juice. Gamgee [1893] reviewed earlier work and concluded that amylase was in the juice, but invertase was mainly in the cells; Bierry [1912] and Bierry and Frouin [1906] thought that maltase was secreted, but invertase was in the cells; Euler and Svanberg [1921], discussing invertase only, thought that it came from cells and Koskowski [1926] that the amylase and invertase found in the juice were both cell-enzymes. Röhmann and Nagano [1903], working on absorption, concluded that cane-sugar and maltose were split partly in the gut lumen but mainly in the mucosa during absorption, and that lactose was not split by the juice and only slightly in the mucosa. Cajori [1933], who combined investigation of the juice with experiments on absorption from the fistula, found that starch was digested by the juice and absorbed from the fistula at about the same rate, but that the juice secreted in a given time only split a small proportion of the cane-sugar and peptone which could be absorbed from the fistula during the same length of time, and he concluded that certain food materials were mainly digested during their passage through the mucosa. Starling [1911] mentioned that a similar view was held by many physiologists. More recently Howell [1926] wrote: "Experiments have shown that this liquid (the intestinal secretion) has little or no digestive action except upon the starches, and it may perhaps be doubted whether it is a true digestive secretion. Extracts of the walls of the small intestine or the juice squeezed from these walls have been found, on the contrary, to contain four or five different enzymes and to exert a most important influence upon intestinal digestion. These enzymes belong probably to the group of endo-enzymes, and are not actually secreted into the lumen of the intestines. While they are not, strictly speaking, constituents of the intestinal juice, nevertheless it is their action on the food which forms the characteristic contribution to the process of digestion made by the glands of the intestinal wall."

(b) *Bacterial Enzymes.*—Leube [1868] noticed that his digest mixtures became acid and found "vibriones" in them. Kühne [1868] discussed the theory that Pasteur's organisms were responsible for digestion, but argued that higher animals must be as capable of producing chemical ferments as the little "torulaces and vibriones." Masloff

or mucoid juice or centrifuged deposit, which would all be likely to contain cell debris, with clear or fluid juice.

Kühne [1868] compared clear fistula juice, which did not digest starch, fat, albumen or meat, with juice obtained by other workers from tied-off loops which, owing, he believed, to pancreatic enzymes or to solid matter, digested those substrates. Tubby and Manning [1892] noticed that starch and cane-sugar were more often digested by the mucous than by the fluid part of the juice from a human fistula. Frouin [1906] found saponification of fats by whole juice from dog fistulæ, and by the centrifuged deposit, but not by centrifuged and filtered juice. Bierry and Frouin [1906] found that at the height of secretion dog fistula juice was turbid owing, they believed, to cell débris separated from the fistula by peristalsis. By first washing out the fistula they were able to collect clear juice. Both turbid and clear juice were centrifuged and passed through a Berkefeld filter. The clear juice digested maltose, not starch or cane-sugar; the turbid juice digested maltose, starch, cane-sugar and trehalose; extracts of intestinal mucosa treated in the same way digested the same four carbohydrates. Jansen [1910] with dog fistulæ found that the mucoid part of the juice was more lipolytic than the supernatant fluid. Koskowski [1926], also working with dogs, compared clear juice, turbid juice, and turbid juice which had been shaken with glass beads to break up the cells; the first two digested starch and cane-sugar feebly, the last strongly.

These all found that the digestive power of turbid or mucoid juice was stronger than that of clear or fluid juice. On the other hand Dobroslawin [1870] found that floccules suspended in water were less amylolytic than filtered juice. Hamburger and Hekma [1902 and 1904] found that both filtered and unfiltered juice digested casein, peptone, starch and cane-sugar, and that both contained enterokinase; their subject was a human patient and it is likely that unfavourable conditions such as a long time or a high temperature during collection might lead to cells breaking up and liberating their enzymes before the juice was filtered; relatively rough chemical tests might then fail to show any difference in the digestive power of the two kinds of juice. The same fallacies may account for the finding of amylase, invertase, erepsin and enterokinase by Hermann and Ribère [1931] in human fistula juice in spite of prolonged and vigorous centrifuging. Foà [1908] took precautions against cell enzymes by centrifuging dog fistula juice as soon as it was collected and later passing it through a bacteriological filter, and it is a pity that his chemical methods do not justify his conclusion that peptone was digested.

Pierce *et al.* [1935] showed, like the earlier authors quoted, that centrifuged juice digested several substrates feebly, but that juice which had been shaken with glass beads to macerate the deposit digested them more strongly. The deposit, which varied in amount and was often considerable, contained a few mucosal cells, but was mainly amorphous; it appeared "like a partially coagulated state of the proteins" and the authors thought it seemed "certain that the solid or semi-solid particles adsorb the enzymes or are the enzyme

In addition, certain comparisons have been made. The duodenal juices obtained from acute cat preparations by different stimuli (sympathetic section, administration of eserine, etc.) have been compared with each other and with juices from other levels of the small intestine produced by the same stimuli. The duodenal juices obtained from permanent fistulæ in five different species of animal have also been compared. The distribution, cellular constitution, and staining properties of Brunner's glands vary from species to species [Florey and Harding, 1933, 1934; Carleton, 1935], which justifies a search for differences in the properties of the secretion. Thirdly, a comparison has been made between juice secreted in response to natural hormonal stimuli alone and in response to nervous and hormonal stimuli together, *i.e.* between juice from denervated and innervated fistulæ. Bowie and Vineberg [1935] showed that in the stomach histamine produced a maximal secretion of HCl but no pepsin, whereas stimulation of the vagus produced both acid and pepsin. Pancreatic secretion also varies according to the stimulus applied. It was thought that changes of the same sort might occur in the enzyme content of the duodenal secretion.

Methods.

The methods used for obtaining juice from acute preparations and for making permanent fistulæ have already been described.

Collection of Juice from Permanent Fistulæ.

The duodenal fistulæ, both transplanted and innervated, secreted spontaneously during digestion in all the five species of animal used and the rate of secretion remained constant for each animal for months. Nothing is known about the methods used by Ponomarew and other Russian workers for collecting juice from duodenal fistulæ. The majority of workers on the jejunum and ileum have inserted a catheter into the fistula to drain it and, by mechanical stimulation, to increase the output of fluid. Thiry [1864] and Röhmann [1887] believed fluid obtained in this way to be the normal output of the intestinal glands, but Boldyreff [1928] stated that it differed from spontaneously secreted juice in having weaker enzymes and an inconstant composition, and believed it to be a mixture of exudate and natural juice. Pavlov [1910] believed that a protective secretion of water was produced, as the kinase content of the juice fell rapidly during a period of mechanical stimulation. Mechanically stimulated juice necessarily contains an abnormal number of epithelial cells and sometimes blood.

By tying a small crucible or pyrex pot over the mouth of a duodenal fistula it is possible to collect spontaneously secreted juice without touching or stimulating the mucosa in any way. This method has

[1882] noticed that solution of fibrin only occurred when the smell of the juice indicated decomposition and microscopy showed bacteria.

Many workers used antiseptics to prevent bacterial action. Masloff used thymol and discussed whether it inhibited the enzyme action he was looking for; Mendel [1896] used thymol or sodium fluoride; Widdicombe [1902] found that thymol effectively inhibited bacterial action; Boldyreff [1906–07] used calomel or thymol; Sawitch [1917] used several different antiseptics separately or together and had identical results with all, and Bierry [1912] passed the juice through a Berkefeld filter or used three different antiseptics together. Bastianelli [1892] took more elaborate precautions, washing out the fistula with thymol before collecting the juice and using sterilised vessels; the juice contained floccules and shreds of mucosa and he found that starch and cane-sugar were digested and that egg-white, fibrin and albumose were not. Miura [1895], when investigating the inverting properties of the intestinal mucosa of animals, ingeniously used the mucosa from stillborn infants as a sterile control; he concluded that inversion by the mucosa was independent of bacterial action. Hamburger and Hekma [1902] cultured the juice from their human patient and inoculated the growth into boiled juice and, after incubating, used the culture or its filtrate as a control to fresh juice; they showed that the culture or its filtrate did not activate trypsin, whereas the fresh juice did.

Bacterial enzymes probably caused digestion, as Masloff suggested, in early experiments, when food substances were left in the juice or even inside the fistula for several days and solid pieces of meat, fibrin or coagulated egg-white were found to be "dissolved." But later experiments lasted a shorter time and were done *in vitro*, and those workers who took steps to lessen bacterial action found no difference in the results of the tests.

Summarising the literature, juice from duodenal, jejunal and ileal fistulæ clearly contains a number of enzymes which digest food substances. The enzymes do not appear to be bacterial, but there is considerable evidence that some of them come from autolysed cells and are not secreted with the fluid part of the juice. Possible exceptions are amylase and perhaps maltase; the source of the enterokinase in the juice has not been thoroughly investigated.

Present Work.

The experiments described in the first part of this paper have given the opportunity to investigate the digestive action of duodenal juice obtained by a variety of experimental methods. Since many samples of juice were almost cell free, while others contained many cells, it has been possible to deduce some of the digestive properties of the secreted fluid as distinct from those of the whole juice.

When a few drops or a loopful of the incubated digest at each pH were cultured on agar slopes, the cultures were usually sterile; sometimes they grew one or two colonies. The presence of colonies bore no relation to the occurrence of digestion, and a sterile digest and one which grew a colony gave identical results when digestion was estimated.

Methods of Testing for Enzymes.

The following buffer solutions were used:—

pH 2–8: Sørensen's buffers [Clark, 1928];
i.e. pH 2, 3, and 4, citrate-HCl;
pH 5 and 6, citrate-NaOH;
pH 7, phosphate;
pH 8, borate-HCl;
pH 8 and 9, veronal-HCl [Michaelis, 1930].

The borate buffer was used at pH 8 for ammonia estimations as the veronal buffer was found to give off small amounts of a volatile base after incubation. The veronal buffer was used for all other methods.

1. *Proteases.*—The substrates were 5 p.c. egg albumen, 3 p.c. gelatine and 5 p.c. casein ("soluble" or Hammarsten's casein from Schering-Kahlbaum). Each experiment was set up at pH 2, 4, 6 and 8 or at pH 2, 5, 7 and 9. In a few early experiments digestion was looked for by estimating increases in amino-nitrogen, but the method was abandoned as the increase would be too small if digestion proceeded only to the poly-peptide stage. In subsequent experiments the digest was treated with trichloracetic acid or colloidal iron and increase in non-precipitable nitrogen was estimated by micro-Kjeldahlisation followed by micro-estimation of ammonia [Conway and Byrne, 1933; Conway, 1935].

2. *Cathepsin.*—For activation, the juice (4 c.c.) was incubated with H_2S water (1 c.c.) at 37° C. for half an hour. The H_2S was then removed *in vacuo* at room temperature and the juice was set up for digestion with gelatine and either albumen or casein at pH 4, 6 and 8 or pH 5 and 7. Increase of non-precipitable nitrogen was estimated as above.

3. *Poly- and Di-peptidase.*—The substrates were 5 p.c. "Difco" bacto-peptone and 1·25 p.c., 3 p.c. or 5 p.c. glycyl-glycine (Hoffmann-La Roche). Each experiment was set up at pH 2, 4, 6 and 8 or at pH 2, 5, 7 and 9. The criterion of digestion was increase in amino-nitrogen, estimated by the semi-micro-method of Van Slyke [1913–14].

4. *Amylase.*—The substrate was 1 p.c. boiled potato starch solution. Each experiment was set up at three or four different H-ion concentrations between pH 4 and pH 9. The criterion of digestion was increase in reducing power. Hagedorn and Jensen's [1923] method for estimating reducing substances was used at first, but later was replaced by Miller and Van Slyke's [1936] method, in which ferro-

been used throughout the present work. As soon as possible after collection the juice was centrifuged fast enough to throw down macroscopic particles, and was immediately poured off from the deposit. Then it was shaken with toluol and put in the ice-chest till it was used, usually the next day.

Appearance of Juice.

Acute juices obtained by vagal stimulation, splanchnicotomy and eserinisation were opalescent and had a small deposit. Post-ganglionic sympathectomy juice was turbid with cells. Acetylcholine juice was turbid with cells and often contained whole villi and blood.

Permanent Fistulæ.—Pig juice was usually clear, sometimes faintly turbid, and there was usually a small deposit after centrifuging. Cat and rabbit juice were almost always water-clear and often there was no visible deposit. Goat juice was clear or slightly cloudy. Dog juice more often than the others contained fine floccules; sometimes they came down completely on centrifuging, but sometimes the finest particles remained behind and made the juice faintly turbid. The juice from any animal might occasionally be tinged yellow or brownish, presumably by blood pigments; very occasionally a few red blood corpuscles were present. There was no difference in appearance or consistency between juice from transplanted and innervated fistulæ. Centrifuged deposits consisted of mucus and disintegrating epithelial cells.

Precautions Against Extraneous Enzymes.

1. *Gastric and Pancreatic Enzymes.*—These were removed in all acute experiments by carefully washing out the loop of intestine with saline, or by throwing away the first few c.c. of juice collected.

2. *Cellular Enzymes.*—The first precaution with permanent fistulæ was never to collect juice until the fistula was perfectly healed, the second was not to touch the fistula during the collection so as to avoid rubbing off any mucosal cells, and the third, which applied to acute and permanent preparations, was to centrifuge off as many cells as possible immediately the juice had been collected.

To show the effect of epithelial cells collections of duodenal and jejunal juice were divided into two parts. One part was centrifuged and to the other small amounts of scrapings of intestinal mucosa were added. Tests for di-peptidase and invertase showed that, whereas the centrifuged juices did not digest the substrates, strong digestion occurred in the samples which contained mucosal scrapings.

3. *Bacterial Enzymes.*—The juice was shaken with toluol as soon as it had been centrifuged, unless it was to be used immediately. Toluol was also added to all digest mixtures except in the few lipase tests which only lasted 6 hours. About 4 drops were added to each c.c. of the mixture.

When a few drops or a loopful of the incubated digest at each pH were cultured on agar slopes, the cultures were usually sterile; sometimes they grew one or two colonies. The presence of colonies bore no relation to the occurrence of digestion, and a sterile digest and one which grew a colony gave identical results when digestion was estimated.

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2. *Cellular Enzymes.*—The first precaution with permanent fistulæ was never to collect juice until the fistula was perfectly healed, the second was not to touch the fistula during the collection so as to avoid rubbing off any mucosal cells, and the third, which applied to acute and permanent preparations, was to centrifuge off as many cells as possible immediately the juice had been collected.

To show the effect of epithelial cells collections of duodenal and jejunal juice were divided into two parts. One part was centrifuged and to the other small amounts of scrapings of intestinal mucosa were added. Tests for di-peptidase and invertase showed that, whereas the centrifuged juices did not digest the substrates, strong digestion occurred in the samples which contained mucosal scrapings.

3. *Bacterial Enzymes.*—The juice was shaken with toluol as soon as it had been centrifuged, unless it was to be used immediately. Toluol was also added to all digest mixtures except in the few lipase tests which only lasted 6 hours. About 4 drops were added to each c.c. of the mixture.

Method of Testing for Lysozyme.

After centrifuging the juice at 5000 r.p.m., serial dilutions were set up against an 18-hour culture of Micrococcus Lysodeikticus suspended in saline, the opacity of the suspension being equivalent to No. 4 Ba sulphate standard. The titre was taken as the highest dilution at which complete lysis, read macroscopically, occurred after incubation for 1 hour at 37° C. Some tests were read again after standing for 12 hours at room temperature. The method is essentially the same as that of Goldsworthy and Florey [1930].

Results.

The tables summarise the enzymes tests on juices obtained by different methods and from different animals. Every test has been entered on the tables, so there is no selection of results. A few qualitative tests which were done at the beginning of the work are included for completeness.

Enzyme Tests on Juices from Acute Preparations (Table I.).

Three complete tests for *protease*, on cat duodenal juices obtained by two different methods, were negative. Three tests on duodenal and two on lower intestinal juice set up at pH 7 only were also negative.

Di-peptidase.—The one test on rabbit duodenal juice was negative. With cat juice, 10 out of 12 duodenal and 2 out of 4 lower intestinal samples were negative. The four juices which gave positive results were exceptionally cellular; one each of these duodenal and lower intestinal samples was obtained by acetylcholine stimulation, which usually brings off villi and fragments of mucosa, and in these particular juices the cellular floccules, instead of being centrifuged out, were chopped to distribute them evenly through the juice, so that it would have been surprising if peptidase had not been present. (The one uncentrifuged acetylcholine juice which did not digest the di-peptide did not contain floccules.) The other two positive results came from duodenal and lower intestinal juices collected after cutting the post-ganglionic sympathetic nerves; these juices were opaque even after centrifuging and were the most turbid juices used in any experiment. In these four positive experiments from 20 p.c. to 80 p.c. of the substrate was split; maximum digestion took place at pH 7 once, at pH 8 twice and at pH 9 once, *i.e.* in agreement with the established optimum pH for "intestinal erepsin."

Poly-peptidase.—Eleven juices were tested, 9 from cat duodenum and 2 from cat jejunum or ileum. The four samples which digested the di-peptide digested peptone to a lesser extent (between 9 and 22 p.c. splitting of the substrate); and in addition another acetylcholine duodenal juice gave 14 p.c. digestion in spite of having been centrifuged.

cyanide is titrated directly with ceric sulphate in the presence of an oxidation-reduction indicator.

5. *Invertase.*—The tests were set up at the same time as those for amylase, and the same methods were used. The substrate was 5 p.c. cane-sugar and the experiments were set up at several H-ion concentrations between pH 5 and pH 9.

In all the above tests, digest mixtures consisted of equal quantities of juice, substrate solution and buffer. Incubation lasted usually for 16 hours, occasionally for 24 or 48 hours. Zero values for each experiment were obtained by estimating samples taken immediately after mixing juice and substrate. Blank experiments were carried out (1) with boiled juice and substrate, (2) with substrate and buffers, (3) with juice and buffers, incubated for the same length of time.

6. *Lipase.*—The juice was brought to pH 7 (blue-green to brom-thymol-blue) with 0·1 N HCl. 1 c.c. of neutralised juice (or 0·5 c.c. if more was not available) and 0·25 c.c. of triolein were mixed with 0·75 c.c. of 0·5 p.c. Na taurocholate or with water. Blank experiments with juice and triolein separately were also set up. The bottles were shaken mechanically in the incubator for 6 or 16 hours, then the contents were poured into beakers and the bottles washed twice with 3 c.c. of absolute alcohol, the washings being added to the digests. Increase in acid was estimated by titrating with 0·01, 0·02 or 0·1 N NaOH, using brom-thymol-blue as indicator.

7. *Enterokinase.*—Pancreatic juice was obtained by cannulating the pancreatic duct of a cat, eserinising, and then injecting acetylcholine subcutaneously and secretin intravenously. The trypsin in such juice is inactive and will remain so for weeks or months in the ice-chest.

Equal quantities of pancreatic and intestinal juice were incubated together for half an hour or more, then 1 c.c. of the mixed juice was set up for digestion with 0·5 c.c. of 5 p.c. egg albumen and 0·5 c.c. of buffer, pH 8 or pH 9. The amino-nitrogen was determined immediately after mixing and again after 16 hours' incubation. Controls were set up with pancreatic and intestinal juice separately and with pancreatic juice treated with an efficient activator supplied by Dr. C. L. G. Pratt.

Tests for enterokinase were also made on some samples of juice by utilising the capacity of pancreatic juice to clot milk in the presence of active trypsin [Mellanby, 1912–13; Kunitz and Northrop, 1933]. The results obtained by the two methods were identical.

Estimations of Neutralising Power of Juices.

The estimations were made on fresh juice as soon as it had been collected. The juice was titrated with standard HCl against brom-thymol-blue or phenolphthalein.

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Source of juice.	Method of obtaining juice.	Enzymes sought.							
		Proteinases.		Peptidases.		Carbohydrases.		Lipase.	Entero-*kinase*.
		Albumen.	Casein.	Peptone.	Glycyl-glycine.	Starch.	Cane-sugar.	Triolein.	Albumen.
Cat Duodenum	Vagal stimulation	none none (pH 7 only) *	. . . .	trace trace none	none none none	40% . .	none . .	none none*	present present
	Vagal stimulation and splanchnicotomy	. .	. .	. .	none	40% 15%	none		
	Splanchnicotomy	none	none	none	none	50%	trace	none	present
	Post-ganglionic sympathectomy	. .	. .	21%	38%				
	Eserine	none (pH 7 only) *	none (pH 7 only) *	trace *	none * none none	present* 40% . .	trace	none * none * none	present present
	Acetylcholine	. .	. .	trace * none 14%	60% * none none *	present . .	present . .	trace * none *	present
Cat Jejunum and ileum	Splanchnicotomy	. .	. .	. .	none				
	Post-ganglionic sympathectomy	. .	. .	12%	80%	70%			
	Eserine	none (pH 7 only) *	none (pH 7 only) *	. .	none	46%	present trace	. . . .	present present
	Acetylcholine	. .	. .	22% *	20% *	present*	present*	trace *	
Rabbit Duodenum	Loop tied off under nembutal. No stimulation	. .	. .	. .	none	. .	. .	. .	present

NOTES.

1. Percentage = digestion equivalent to % of theoretical possible splitting of substrate.
2. Trace = less than 10% splitting.
3. * = juice not centrifuged.
4. Each entry = one test.
5. Results on the same vertical line are all from different samples of juice.
6. Results on the same horizontal line are not necessarily from the same sample of juice.

Maximum digestion took place at pH 7 twice, pH 8 twice, and pH 9 once. Three out of the other 6 duodenal samples gave a trace of digestion (less than 10 p.c.), but as the corresponding tests on the di-peptide were negative a technical fault was suspected though it could not be proved; in these three tests maximum digestion took place at pH 7 each time.

Amylase was present in every one of the 10 juices tested, 7 from cat duodenum and 3 from cat jejunum or ileum. Usually 40 p.c. or more of the starch was digested. In 7 quantitative tests maximum digestion took place at pH 7 four times, at pH 6 and 7 once, equally at pH 5, 6, and 8 once and at pH 4 in the test on post-ganglionic sympathectomy juice from the lower ileum. Digestion took place over a wide range of H-ion concentration, and between pH 5 and pH 8 the differences in the amount of splitting were small.

Invertase.—The same two acetylcholine juices which digested the di-peptide digested cane-sugar (qualitative). Of six other cat juices, two duodenal samples gave a trace of digestion and two gave none, and one lower intestinal sample gave a trace of digestion and another was qualitatively positive. In the three quantitative positive tests maximum digestion took place at pH 7 twice and at pH 5 once.

Lipase.—The same two acetylcholine juices which digested the di-peptide slightly digested fat. Seven other tests, all on cat duodenal juice, were negative; one of these negatives came from uncentrifuged acetylcholine juice, but this was the exceptionally acellular juice which also failed to digest peptides.

Trypsin was activated by every one of 9 juices tested, showing that *enterokinase* was consistently present. The juices were obtained by 5 different methods; one came from rabbit duodenum, 5 from cat duodenum, and 3 from cat jejunum or ileum.

Enzyme Tests on Juices from Permanent Duodenal Fistulæ. (Tables II. and III.)

Denervated (transplanted) duodenal fistulæ were made in 3 pigs, 3 cats, 2 rabbits, and 1 goat, and juices were tested for enzymes before and after the pedicle was cut. Innervated fistulæ (in which the muscle of the duodenum remained in continuity with that of the stomach and the mesentery was left intact) were made in 4 pigs, 2 cats, and 2 dogs. The results of the tests were similar in all animals and in both kinds of fistulæ.

Proteases were tested for in 36 experiments and *poly-* and *di-peptidase* in 33; the results in every case were negative. *Amylase*, on the other hand, was present in every one of the 18 juices tested; pig, dog and rabbit juice split from 45 p.c. to 80 p.c. of the substrate in 9 quantitative tests and cat juice slightly less (20 p.c. to 50 p.c.). As in the acute experiments, maximum digestion occurred at pH 5, 6 or 7 and the

Table II.—Digestion of Substrates by Juice from permanent Duodenal Fistulæ: (1) Pigs.

Type of fistula.	No. of pig.	State of pedicle (in transplanted fistulæ).	Enzymes sought.								
			Proteinases.			Peptidases.		Carbohydrases.		Lipase.	Entero-kinase.
			Albumen.	Gelatine.	Casein.	Peptone.	Glycyl-glycine.	Starch.	Cane-sugar.	Triolein.	Albumen.
Transplanted	1	not cut	none	none	none	none none	none none	present present present	none present	none	
		cut	none	none	..	..	..	..	..	..	none none
	2	not cut	..	..	..	..	..	65%	..	..	none
		cut	none	..	none none	none ..	none ..	70% ..	none ..	none none	present present
	3	cut	none	..	none	none	none	60%	none	none	present
Innervated	4	..	none	none	none	none	none	present	..	..	present present
	5	..	none	..	none	..	none	45%	none	trace trace	present
	6	..	none	..	none	none	none	80%	trace	trace	present
	7	..	none	..	none	none	none	55%	trace	none none	present present

For explanatory notes see Table I.

TABLE III.—DIGESTION OF SUBSTRATES BY JUICE FROM PERMANENT DUODENAL FISTULÆ: (2) DOGS, CATS, RABBITS, GOAT.

Type of fistula.	Animal.	State of pedicle (in transplanted fistulæ).	Enzymes sought.									
			Proteinases.			Peptidases.		Carbohydrases.		Lipase.	Entero-kinase.
			Albumen.	Gelatine.	Casein.	Peptone.	Glycyl-glycine.	Starch.	Cane-sugar.	Triolein.	Albumen.
Innervated	Dog 1	..	none	none	none	none	none	80%	13%	none	present
Transplanted	Dog 2	..	none	none	none	none	none	75%	trace	none	present
	Cat 1	not cut	..	..	..	..	..	..	..	..	present
		cut	..	..	..	..	none				
	Cat 2	cut	..	..	..	none	none	20%	none	none	present present present
	Cat 3	cut	none	..	none	none	none	38%	none	none	present present
Innervated	Cat 4 2–4 weeks after operation	..	none	..	..	none	none	present	..	..	present present
	6 months later	..	none	..	..	..	none	50%	none	none none	present
Transplanted	Cat 5	..	none								
	Rabbit 1	not cut	none none	..	..	..	..	..	..	..	present
	Rabbit 2	cut	none	..	..	none	none	70%	trace	trace	none none
Transplanted	Goat	not cut	none	none	none	none none	none none	trace ..	none trace	trace trace	

For explanatory notes see Table I.

differences within this range were small. A trace of *invertase* was found in 7 juices out of 15, the greatest digestion being 13 p.c. splitting after incubation for 48 hours (dog 1). The H-ion concentration at which maximum digestion occurred ranged from pH 5 to pH 7, as in the tests for amylase. Slight *lipase* action, greater in the presence of sodium taurocholate, was found in 6 out of 18 juices.

Enterokinase was present in all juices except those of one rabbit and one pig and the first of three samples from another pig; no explanation can be offered for its absence from these juices. In the positive experiments there was no significant difference in tryptic activity between test and activated control. In the milk-clotting test there was no difference in clotting time between test and control. The positive results were obtained at various times from 1 week to 6 months after making the fistulæ.

Tests for Cathepsin.

Taylor *et al.* [1938] found that casein was digested, mainly to proteoses, by normal human gastric juice acting at pH 7·4. There has been no evidence of a similar action by duodenal juice in the present work. A few juices from permanent fistulæ were activated with H_2S, and those also gave no evidence of a proteolytic action at H-ion concentrations between pH 4 and pH 8.

TABLE IV.—NEUTRALISING POWER OF JUICES FROM PERMANENT DUODENAL FISTULÆ.

Animal and preparation.	Neutralising power of juice in c.c. 0·1 N HCl.
Pig 3—Transplanted fistula	0·23
Pig 7—Innervated fistula	0·31
Dog 1—Innervated fistula	0·18
„ (another sample)	0·32
Dog 2—Innervated fistula	0·34
„ (another sample)	0·22
Cat 1—Transplanted fistula	0·35
Cat 2— „ „	0·40
Cat 3— „ „	0·42

Estimations of Neutralising Power.

Duodenal juice obtained by a variety of stimuli from acute cat preparations neutralised 0·2 c.c. of 0·1 N HCl per c.c. of juice twelve times and 0·3 c.c. seven times. One sample had no neutralising value and one neutralised 0·1 c.c. of acid. When the upper (Brunner's gland area) and lower parts of the duodenum were cannulated separately, the

juice from the upper part gave a value of 0·2 c.c. of acid and the juice from the lower part had no neutralising value (three experiments, vagal stimulation). Ileal juice was examined three times. A sample collected after eserine stimulation gave a value of 0·15 c.c. of acid, and two successive samples from a cat after post-ganglionic sympathectomy had the unexpectedly high value of 0·60 c.c. of acid.

The results of the few estimations on duodenal fistula juices (Table IV.) agree with those of Florey and Harding [1934]. Cat fistula juice has a slightly greater neutralising power than the juice from acute cat preparations and also than fistula juice from the dog and the pig.

Lysozyme Tests. (Table V.)

Cat juice contained no lysozyme (4 samples) and dog juice produced no immediate lysis and only partial lysis after standing overnight (3 samples). Pig juice, on the other hand, produced complete lysis at titres from 1 : 256 to 1 : 8192 after incubation for one hour (6 samples). Florey and Harding [1933] found weak lysozyme in rabbit duodenal juice, but in the one sample tested in the present series none was present.

Table V.—Lysozyme Titres of Juices from permanent Duodenal Fistulæ against M. Lysodeikticus.

Animal and preparation.	Titre : complete lysis except where stated otherwise.	
	After 1 hour at 37° C.	Same after 12 hours at room temperature.
Pig 2—Transplanted fistula	1 : 8000 (highest dilution used)	
Pig 3— ,, ,,	1 : 256	1 : 512
Pig 6—Innervated fistula	1 : 512	
,, (another sample)	1 : 512	
Pig 7—Innervated fistula	1 : 8192 (highest dilution used)	
,, (another sample)	1 : 256	1 : 512
Dog 1—Innervated fistula	No lysis	Partial lysis at 1 : 128
,, (another sample)	,,	,, ,, 1 : 32
Dog 2—Innervated fistula	,,	,, ,, 1 : 128
Cat 1—Transplanted fistula	No lysis	
,, (another sample)	,,	
Cat 2—Transplanted fistula	,,	
Cat 6—Innervated fistula	,,	
Rabbit 2—Transplanted fistula	No lysis	

We are indebted to Mr. L. A. Epstein for doing these tests.

Discussion.

The Control of Secretion.—In reviewing the experimental findings it is convenient to consider first the control of the secretion in the upper part of the duodenum, which contains Brunner's glands. An abundant secretion of alkaline mucoid juice is produced from this part in acute cat preparations by (1) stimulating the vagi, (2) cutting the splanchnic nerves in the thorax, (3) administering eserine either alone or with acetylcholine. A scantier secretion is produced by the intravenous injection of material containing secretin. The consistency of the juice obtained by any of these methods is indistinguishable from that of juice from permanent duodenal fistulæ, though the cellular content varies with different methods of stimulation. It has been confirmed that the secretion of permanent transplanted duodenal fistulæ in cats is controlled by a hormone, as after 24 hours' starvation they are dry but secrete well after feeding. Similar denervated fistulæ in pigs react in the same way. The clear-cut results of these experiments leave little doubt that the secretory activity of the first part of the duodenum is under both nervous and hormone control.

In accordance with the current view on the chemical transmission of parasympathetic stimulation it may be supposed that acetylcholine is liberated either in the enteric plexuses or in the neighbourhood of the secreting cells or both, and that either the acetylcholine may be formed by vagal activity or "liberated" by the removal of sympathetic inhibition, or the breakdown of acetylcholine formed in the gut plexuses in the absence of stimuli may be prevented by eserine. Experimentally, the secretory effects of vagal stimulation, sympathectomy and eserine injection are all inhibited by atropine, which is evidence that they may act through a common mechanism. It seems certain that the juice obtained from the duodenum after cutting the splanchnic nerves is not a transudate from the blood, as it contains the "soluble" mucin typical of Brunner's gland secretion. The inhibition of the "paralytic" secretion by atropine and anæsthetics also supports this view.

The relative importance of the extrinsic nerves and the hormone mechanism in producing duodenal juice is difficult to assess. In acute experiments much more secretion can be produced by stimulating the vagus than by injecting secretin. In permanent preparations, on the other hand, transplanted fistulæ and fistulæ with the extrinsic nerves intact both stop secreting during starvation and start secreting immediately feeding begins (cats and pigs); both secrete about the same amount of juice and the composition of the juice from the two kinds of fistulæ appears to be identical.

The profoundly depressing effect of anæsthetics on intestinal secretion is probably one of the reasons why the control of the duodenal secretion has not previously been analysed. It is noteworthy that the

experiments in which Savitch obtained secretion, apparently from the whole intestine, in response to vagal stimulation, were done on decapitate cats.

In our experiments the vagus has had less influence on the rest of the small intestine than on the duodenum. Vagal stimulation produces a watery secretion from a small part of the duodenum just distal to the Brunner's gland area. In two experiments stimulation of the vagus after splanchnic section caused secretion from the jejunum and ileum. The failure of the jejunum and ileum to secrete in response to simple vagal stimulation does not seem to have been due to inadequate stimulation, as in all experiments good gastric and duodenal secretion occurred.

Though section of the splanchnic nerves "liberated" the duodenum only, section of all preganglionic nerves caused secretion from the whole of the small intestine. Certain experiments support the idea that there is a "centre" in the spinal cord from which inhibitory impulses pass to the secretory mechanism of the gut. If the influence of this centre is removed by section of all preganglionic fibres the ganglia at the root of the mesentery take over inhibitory powers, their extirpation being followed by secretion. It is possible that the inhibitory effect of the splanchnics is so powerful that, under the present experimental conditions, vagal stimulation is unable to overcome it except in the duodenum.

Generally, to mimic vagal action by pharmacological means it is necessary to eserinise an animal and then to give acetylcholine. Eserine alone, however, causes intestinal secretion, though no other evidence of cholinergic action (*e.g.* salivary or tracheal secretion) appears. The addition of acetylcholine causes intense spasm of the intestine and epithelial cells and even intact villi are shed, while at the same time salivary secretion occurs.

The general conclusion appears to be justified that the gut, probably in its rich nerve plexuses, makes a relatively large quantity of acetylcholine, which is prevented from acting by the sympathetic nerves. The inhibition can be overcome experimentally by the administration of eserine or by section of the appropriate sympathetic nerves.

Although the effects of nerve stimulation and section can be demonstrated with regularity, there is as yet no evidence as to how they are integrated with intestinal function. For example, Thiry fistulæ of the jejunum and ileum, which retain their sympathetic innervation, do not, according to most observers, secrete juice during digestion. The small amount of "periodic" secretion [Boldyreff, 1928] occurs during starvation, not after feeding. One would have expected that if there were a general lowering of sympathetic "tone" during digestion secretion would occur in the fistulæ. The relation of the effects of local irritation or stimulation of the mucosa to the extrinsic nerves is even less under-

stood, but there is good evidence that the jejunum and ileum deprived of their extrinsic nerves *can* produce juice when stimulated by a catheter [Ivy *et al.*, 1927; Nassett *et al.*, 1935].

Enzymes.—The enzyme content of duodenal juices produced in a variety of ways and from different species of animals has been investigated. In the introduction to Part II. various authors are mentioned who found protease, amylase, invertase, lipase and enterokinase in juice from duodenal fistulæ. The available writings of the Russian workers give no indication of the cellular content of their juices and possibly they, like many of the workers with jejunal and ileal fistulæ, were actually testing a mixture of pure secretion and cells. We have attempted, by eliminating cellular and bacterial enzymes as far as possible, to estimate the digestive properties of the actually secreted portion of duodenal juice.

Two groups of enzymes appear to be absent from cell-free juice—the proteases and the peptidases. No protease was found in any juice, and no peptidase in any juice from a fistula. Certain juices from acute preparations contained poly- and di-peptidase, but, with the exception of traces of poly-peptidase in three samples, there was a clear connection between peptide digestion and the high cell-content of the juices. Many samples of juice gave little or no deposit on centrifuging, so that nothing was removed to which enzymes might have been adsorbed, as Kestner [1930] and Pierce *et al.* [1935] argued.

Two other enzymes, invertase and lipase, which were present in cellular juices, sometimes appeared also in traces in other juices, but the splitting they produced was insignificant.

Two enzymes, amylase and enterokinase, were present in greater amounts. Amylase was present in every sample tested and usually split from 40 p.c. to 80 p.c. of the substrate. Waldschmidt-Leitz [1929] quoted work showing that starch was only digested to about 75 p.c. of the theoretical maximum splitting unless the products of digestion were removed; in 24 hours, therefore, many samples of juice produced something approaching maximum digestion. In his experiments on fistulæ from lower levels of the small intestine, Cajori [1933] found that amylase was the only enzyme in the juice whose digestive action was strong enough to keep pace with the absorption of the substrate; he suggested that it might come from the same source as the amylase of the blood and other body fluids.

In addition to amylase, an enterokinase capable of fully activating trypsin was found in nearly every sample of juice. Since its discovery by Schepowalnikow [Falloise, 1904; Pavlov, 1910; Babkin, 1928] there has never been any doubt of its presence in jejunal and ileal juice, but the only author to mention its presence in duodenal juice was Pavlov, who believed that the function of activating trypsin belonged more to the duodenal juice than to juice from lower levels of the small intestine.

Acute experiments gave unsatisfactory material for comparative tests. The comparison which we had hoped to make between the secretion of the duodenum and that of other levels of the small intestine proved hard to make, as the jejunum and ileum do not secrete in response to vagal stimulation, and other stimuli produce juice which is often very cellular. The comparison between the duodenal secretion produced by different stimuli in one species was also difficult, as the quantity and cellular content of the juice varies with the stimulus. Provided, however, that juice free from cells was obtained, no differences were found between the different methods of stimulation, and the few satisfactory tests on jejunal and ileal juice showed that, as in the duodenal secretion, amylase and enterokinase are present and di-peptidase absent.

The juice from permanent fistulæ, on the other hand, was consistently free from gross cellular contamination, so that the comparison between the duodenal secretion of five different species of animals, and between the secretion of transplanted fistulæ and those with their nerve supply intact, was satisfactory. There is no difference in the digestive enzymes of any of these secretions, though there are species differences in lysozyme content and possibly in neutralising power. Nasset *et al.* [1935] found no qualitative difference in the physical and digestive properties of juice from transplanted intestinal fistulæ before and after cutting the pedicle and we, with duodenal fistulæ, have found none.

The results of these enzyme investigations, which have been done by standard chemical methods and with the necessary precautions, conflict with those of Ponomarew [1902] and of Florey and Harding [1934], since, within the limits of the present investigation, the only enzymes constantly present in duodenal juice are enterokinase and amylase. Florey and Harding [1935 *a*] discussed the evidence that the mucus secreted in the duodenum was a product of Brunner's glands. Possibly the whole of the secretion which flows spontaneously from a permanent duodenal fistula comes from Brunner's glands or possibly part is contributed by the crypts of Lieberkühn. The only important enzyme action of the secretion appears to be the activation of trypsin, and this it shares with the secretion from other levels of the small intestine: the specific mucin content of the juice, however, and its alkalinity make it an admirable medium for protecting the first part of the duodenum against the acid gastric juice.

The close relation between di-peptide digestion and cell content in duodenal juice, and in the few satisfactory experiments on jejunal and ileal juice, leads us to believe that the peptidases (and the same probably applies to invertase and lipase) are endo-enzymes, in the sense that they are not secreted into the intestinal lumen in solution. Linderstrøm-Lang [1939] reviews recent work and considers that peptidases are shown to be typical endo-enzymes and he asks for reconsideration of the

question whether peptidase in the lumen of the duodenum may not originate from autolysed mucous cells. Our failure to demonstrate significant amounts of peptidase in cell-free duodenal juice supports his views. There can be no doubt that there is a constant and considerable desquamation of epithelial cells into the lumen of the intestine, shown, for instance, by the large number of mitotic figures in the crypts of Lieberkühn, and by the presence of cells in the juice from a fistula, which is free from the trauma even of food passing over. Endo-enzymes may be liberated in the intestinal lumen by the breaking up of these cells and so act on the food products before they are absorbed, as well as during their passage through the intestinal mucosa.

The balance of evidence is that juice obtained by experimental methods from the jejunum and ileum is an alkaline fluid which, like that from the duodenum, contains only two secreted digestive enzymes —amylase and enterokinase. There is no satisfactory evidence whether such experimental juice has the same properties as the *succus entericus* secreted during the passage of chyme along the intestine, but it may be assumed that during digestion a fluid is secreted which has other functions beside that of contributing enzymes. It is well recognised that water and salt solutions are very rapidly taken up from the small intestine, and we have shown in this paper that duodenal secretion itself can be absorbed from the jejunum. It may be necessary for a constant secretion of fluid to take place from the crypts of Lieberkühn to keep the food particles in suspension while they are attacked by the pancreatic enzymes, and as the products of digestion are absorbed water and salts go with them. One may therefore envisage a circulation of fluid during active digestion, the secretion passing out from the crypts of Lieberkühn into the intestinal lumen and back into the villi.

Summary.

1. Stimulation of the vagus nerves in decerebrate or decapitate cats causes a secretion from the duodenum which comes principally from the Brunner's gland area.

2. No clear evidence has been obtained that vagal stimulation causes secretion from the jejunum or ileum.

3. Cutting the greater splanchnic nerves in the thorax causes secretion from the duodenum, but not from the lower levels of the small intestine.

4. Cutting all the preganglionic sympathetic fibres causes secretion from all the small intestine.

5. The ganglia of the solar plexus exert an inhibitory influence on secretion after section of the preganglionic fibres.

6. The subcutaneous administration of eserine causes secretion from all the small intestine.

7. The concomitant administration of eserine and acetylcholine may cause extreme damage to the intestinal mucosa.

8. Stimulation of the Nervi Erigentes has no influence on the secretion of the small intestine.

9. Histamine has no influence on the secretion of the small intestine.

10. Certain anæsthetics abolish or considerably reduce secretory activity produced by the above methods.

11. Atropine also abolishes it.

12. Confirmation has been obtained of the presence of a secretory hormone acting on the first part of the duodenum in cats. A similar hormone has been shown to be present in pigs. In dogs, innervated fistulæ cease to secrete after 24 hours' starvation and recommence soon after feeding.

13. Duodenal secretion obtained experimentally contains amylase and enterokinase. It does not contain protease or peptidase. It sometimes contains traces of invertase and lipase, but their digestive power is insignificant.

14. The duodenal secretions of five different species of animal and of both denervated and innervated fistulæ have the same digestive properties.

15. In a few experiments no differences were found between the digestive properties of secretion from the duodenum and from other levels of the small intestine.

16. Juices from the same level of the small intestine secreted in response to different stimuli show differences in digestive properties which are due to cells and not to differences in the secreted fluid.

Dr. E. Chain has kindly advised on the conduct of the enzyme investigations. We are glad to acknowledge the technical help of J. Kent and A. Sale. We are indebted to the Government Grants Committee of the Royal Society and to the Nuffield Trust for grants towards expenses. One of us (R. D. W.) was also in receipt of a personal grant from the Nuffield Trust.

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THE LIBERATION OF A SLOW-REACTING SMOOTH MUSCLE-STIMULATING SUBSTANCE IN ANAPHYLAXIS.

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IN recent experiments carried out in this laboratory [Feldberg and Kellaway, 1938; Feldberg, Holden, and Kellaway, 1938; Trethewie, 1939] evidence has been brought forward which indicates that the contractions of smooth muscles caused by different venoms are not their direct effects nor are they wholly brought about by liberation of histamine. There is formed from tissue constituents by enzymic action of the venoms a "slow-reacting smooth muscle-stimulating substance" which may largely determine the nature of these responses. It was suggested that a similar mechanism might be involved in the reaction of smooth muscles to other injurious stimuli. The experiments recorded in this paper were undertaken to see if this hypothesis applies to the antigen-antibody reaction of anaphylaxis. If this were so, many of the difficulties inherent in the theory that the anaphylactic contraction of smooth muscle is wholly attributable to the liberation of histamine would be removed.

In order to find out whether a muscle-stimulant substance similar to that formed by venoms was formed or liberated during the antigen-antibody reaction and to which the anaphylactic contraction of smooth muscle could be attributed we used both an indirect and a direct approach. Various states of decreased excitability were brought about in smooth muscle by different means with the idea of obtaining conditions under which the responses to histamine, to antigen, and to the substance formed by venoms were affected differently in order to see whether the anaphylactic contraction disappeared together with the response to histamine or with that to the substance formed by venoms. We have also attempted to obtain direct evidence for the appearance of a "slow-reacting muscle-stimulant substance" in perfused tissues and in tissue extracts from sensitised organs treated with antigen.

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water and then either boiled and tested or taken to dryness over sulphuric acid *in vacuo* at room temperature for 24 hours. The powder was ground up, extracted with Tyrode solution, centrifuged, and the sediment re-extracted. The second extract was added to the first and the combined extracts made up to a volume so that 0·2 c.c. contained the equivalent of 5 mg. of tissue. When tested on the gut the perfusate as well as the various extracts were left in contact with the muscle for 10 to 15 seconds. In several experiments the antigen was not injected, but extracts prepared by grinding the tissue with silica sand in Tyrode solution were incubated with antigen (25 to 100 mg. albumin per gram of fresh tissue) at 37° C. for 15 to 30 minutes and then either boiled and tested or taken to dryness and re-extracted as described above before testing.

Results.

A. *The Isolated Jejunum of the Guinea-Pig.*

(i) *The Anaphylactic Response.*—The isolated jejunum of the guinea-pig sensitised to crystalline egg albumin gives a sharp contrac-

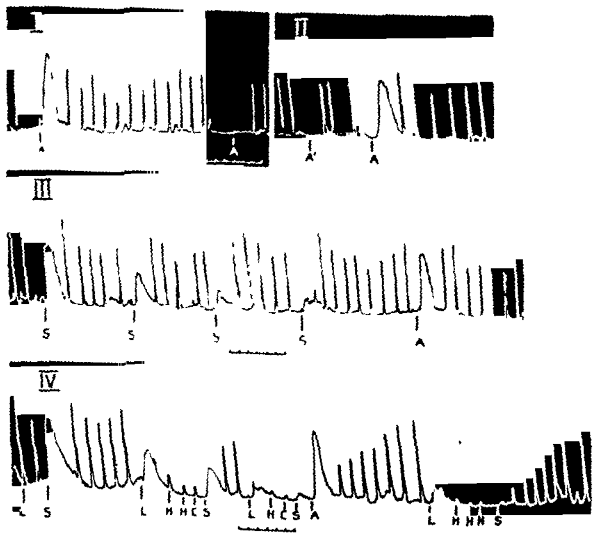

Fig. 1.—Responses of 4 pieces of jejunum from a sensitised guinea-pig. At A, 2 μg., and at A′, 0·2 μg. egg albumin; at S, 2 mg. S.R.S.; at L, 4 mg. lysocithin (latency 20 seconds); and at C, 0·06 μg. acetylcholine. The unlettered contractions and those lettered H in IV are to 0·1 μg. histamine. Time in minutes. Details in text.

tion to the anaphylactic antigen after a latency which varies with the sensitiveness of the gut and the size of the test dose. The size and shape of the observed contraction also depend upon the same two factors. After the antigen has been in contact with the gut for

Methods.

The experiments were carried out on guinea-pigs. Animals weighing 110–150 g. were sensitised by subcutaneous injection of 15 mg. crystalline egg albumin in 0·5 c.c. sterile saline solution and used for experiment usually 3 to 7 weeks later.

The anaphylactic contraction of smooth muscle was studied on pieces of jejunum suspended in a 5-c.c. bath of oxygenated Tyrode solution and in a few experiments on horns of virgin uteri suspended in a similar bath of oxygenated anaphylactic Ringer's solution (NaCl 0·9, KCl 0·042, $CaCl_2$ 0·012, $NaHCO_3$ 0·05, glucose 0·1 p.c.). The antigen was allowed to remain in contact with the gut for 1 minute and with the uterine horn for 2 minutes. The latencies of the responses were measured with a stop-watch. For comparison, histamine and the slow-reacting muscle-stimulant substance formed by venoms, and in a few cases the venom of the cobra (*Naia naia*), were added to the bath. Histamine was used as dichloride, and the amounts given in the text and in the figures refer to the salt and not to the base. Decreased excitability of the muscle was produced by slow-reacting substance (made by the action of cobra venom on egg yolk), lysocithin, photodynamic action, histaminase (Torantil), CO_2, and by the toxin of *Cl. welchii* type A. The lysocithin and slow-reacting substance were prepared for us by Mr. H. F. Holden. In the experiments with photodynamic action 1 mg. sodium hæmatoporphyrinate was added to the bath and left in contact with the muscle for 1 minute. The muscle was then irradiated by two 60-W. lamps at 13 cm. for 12 to 16 minutes.

To obtain direct evidence of the appearance of a slow-reacting muscle-stimulant substance the lungs and the small intestine of the sensitised animals were perfused with Tyrode solution. The lungs were perfused through the pulmonary artery as described by Feldberg and Kellaway [1937], and the intestine through the descending thoracic aorta. In these experiments the abdominal aorta was ligated below the point of origin of the superior mesenteric artery; the renal arteries and the lesser omentum were tied after opening the portal vein. The large bowel and stomach were excised after ligating their mesenteries. The contents of the small bowel were washed out with a litre of Tyrode solution through a cannula introduced into the duodenal stump. The venous effluent was collected in successive samples before and after the injection of the antigen (20 or 40 mg. egg albumin in 0·2 c.c.) and tested on the normal guinea-pig's jejunum. In addition, extracts of the tissues were made. In the experiments on the lungs one side was used as control; in the experiments on the intestine pieces of the perfused jejunum of about 6 to 7 cm. in length were removed before the injection of antigen and used for control extraction. The extracts were made by grinding the tissue with silica sand in Tyrode solution or distilled

latency when the initial dose was 2 μg. was 15 seconds, but after failure to respond to 0·2 μg. it was 30 seconds. In fig. 2, I and VI are the responses to 4 μg. egg albumin from two pieces of intestine from a guinea-pig 29 days after the sensitising dose. The gut in VI had been kept some hours in Tyrode solution at room temperature before testing. The latencies of these two responses were 40 and 70 seconds. In fig. 4, I, the anaphylactic response of a piece of jejunum from a guinea-pig 23 days after the sensitising injection was to 4 μg. egg albumin and the latency 19 seconds, and in fig. 5, I, from a guinea-pig 24 days after the sensitising injection the response was to 1 μg. of antigen and the latency 15 seconds. The response in fig. 4 shows an unusually great after-depression of excitability.

(ii) *The Anaphylactic Response after Repeated Doses of S.R.S.*—Feldberg, Holden, and Kellaway [1939] have shown that a substance with a stimulating effect upon the gut is formed by the action of venoms on egg yolk, lecithin, and perfused organs. This substance, unlike lysocithin, can be extracted with acetone. A substance with similar activity is formed by the action of trypsin on egg yolk [Holden [1]]. The contraction caused by these substances has a short latency and is less rapid than that produced by histamine. Relaxation is gradual when the Tyrode solution in the bath is changed. We shall therefore call the substance or substances responsible for this slow-reacting effect "slow-reacting substance" or S.R.S. The response of the isolated jejunum of the guinea-pig to S.R.S. is similar to that caused by snake venoms or by the anaphylactic antigen in the sensitive gut, save that the latency is shorter than in an anaphylactic response of similar magnitude, and the excitability of the muscle seldom shows the phase of depression which is regularly seen after snake venom and irregularly in anaphylaxis. By repeated administration of large doses of S.R.S. the stimulating effect becomes smaller and the intestinal muscle can be desensitised, though not completely, to S.R.S., whereas the excitability to histamine or acetylcholine remains unimpaired. After such treatment the anaphylactic response is still obtained although it is slightly but definitely reduced. These results are illustrated in fig. 1, III and IV, and fig. 2, V. Fig. 1, III, shows the responses (at S) to four successive doses of 2 mg. each of crude S.R.S., prepared by acetone extraction of egg yolk treated with venom. The contractions progressively diminished and the latencies lengthened; these were 7, 7, 10, and 12 seconds respectively. A subsequent anaphylactic response to 2 μg. antigen with a latency of 30 seconds is shown at A. The previous treatment with S.R.S. had about the same reducing effect on the anaphylactic response as the desensitising effect of a single previous dose of 0·2 μg. antigen in fig. 1, II. The effect on the anaphylactic response is more obvious when large doses of S.R.S. are given at short

[1] Unpublished communication.

1 minute the Tyrode solution in the bath is changed; the gut now slowly relaxes, and its responses to histamine or to acetylcholine in repeated doses exhibit characteristic after-changes in the excitability of the muscle during a period of about 10 minutes. There is generally first an increase, then a decrease, and finally a return to normal excitability. The anaphylactic reaction of the muscle thus resembles that

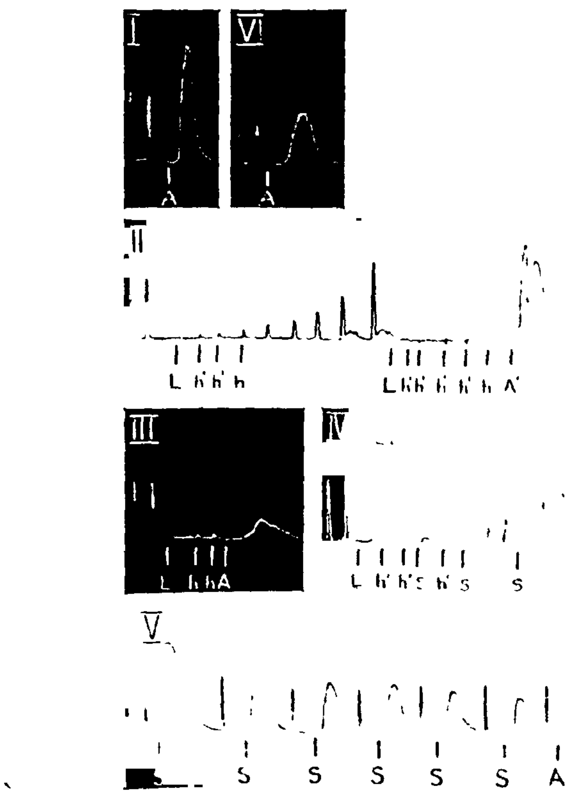

FIG. 2.—Responses of 6 pieces of jejunum from a sensitised guinea-pig. At A, 4 μg., and at A′, 60 μg. egg albumin; at L, 0·5 mg. lysocithin; at S, 1 mg. S.R.S. The unlettered contractions in I, III, V, and VI, and the small contractions *h* in III are to 0·1 μg. histamine. The unlettered contractions and those at *h′* in II and IV are to 0·2 μg. histamine. Time in minutes. Details in text.

to snake venoms [Feldberg and Kellaway, 1937], though the after-depression of excitability is rarely so great as after the contraction caused by venom.

These features are illustrated in figs. 1, 2, 4, and 5. In fig. 1, I and II, are shown the responses of two adjacent pieces of jejunum from a guinea-pig 21 days after the sensitising dose. The gut failed to react to 0·2 μg., but gave a good response to 2 μg. of egg albumin. The

of the jejunum of a guinea-pig weighing 240 g. 35 days after the sensitising injection. In I is the response to 20 μg. egg albumin. This had a latency of 20 seconds. In II, after a dose of 1 mg. of

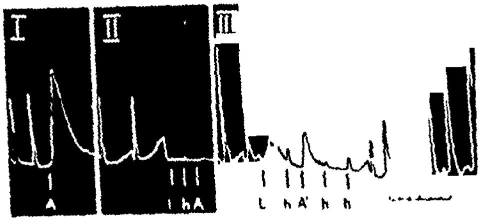

FIG. 3.—Responses of 3 pieces of jejunum from a sensitised guinea-pig. At A, 20 μg., and at A', 200 μg. egg albumin; at L, 1 mg. lysocithin. The unlettered contractions and those at *h* are to 0·1 μg. histamine. The contraction lettered *h'* is to 0·3 μg. histamine. Time in minutes. Details in text.

lysocithin, which caused no contraction and remained in contact with the gut for 1 minute, the gut failed to respond to 0·1 μg. histamine and to 20 μg. egg albumin. In III, after 1 mg. of lysocithin, which caused a small contraction, the gut gave small responses to 0·3 μg. histamine and to 200 μg. egg albumin. The anaphylactic response had a latency of 25 seconds.

(iv) *The Anaphylactic Response after Injury by Photodynamic Action.*—The isolated intestine of the guinea-pig exposed to light after treatment with hæmato-porphyrin exhibits a rapid decline in excitability as measured by its response to histamine [Kellaway and Trethewie, 1939]. This injury may readily be graded, and in its more severe degrees is irreversible. If the response to histamine is not too greatly depressed the anaphylactic reaction, though diminished, can still be elicited, but if the photodynamic action is sufficiently prolonged the anaphylactic response can no longer be obtained. The diminution of the anaphylactic response is roughly paralleled by the depression of the response to histamine. This is illustrated by the experiments of figs. 4 and 5. In II and III of fig. 4 illumination in the presence of hæmatoporphyrin has produced a loss of excitability such as to render the muscle insensitive to 0·1 μg. of histamine or 2 mg. of S.R.S. In this condition the gut gives also only a minute response with 50 seconds latency to 4 μg. egg albumin (in II), but still reacts to larger doses of antigen as well as of histamine. In III there is a large response with 23 seconds latency to 40 μg. antigen, and the muscle gives good contractions to 4 and 6 μg. of histamine. A more complete depression was observed in the experiment of fig. 5, in which are shown the responses of three pieces of jejunum from a guinea-pig 24 days after the sensitising injection. In I the height of the anaphylactic response to 1 μg. egg albumin was matched with the response to 0·5 μg. histamine. In II, after the addition of hæmatoporphyrin and irradia-

intervals and the anaphylactic response is tested shortly after the last dose of S.R.S. In fig. 2, V, six successive doses of 1 mg. S.R.S. were given to a piece of intestine which was not very sensitive to the anaphylactic antigen. The responses had latencies of 10, 20, 27, 28, 28, and 31 seconds and showed a progressive decrease in size. The gut now failed to respond to the threshold dose of 4 μg. of egg albumin (at A).

(iii) *The Anaphylactic Response after Lysocithin.*—Feldberg, Holden, and Kellaway [1939] have shown that lysocithin has no stimulating action on the isolated guinea-pig's jejunum, but that its administration is followed by a period of profoundly depressed excitability to histamine or acetylcholine. We can confirm these observations, but have found in addition some stimulating action when large doses of lysocithin are administered. This effect may have been caused by traces of S.R.S. in the lysocithin preparation or may have been an effect of the lysocithin itself. In that case it was probably caused by liberation of histamine, which has been shown to occur in perfused organs after the injection of lysocithin.

During the period of depressed excitability the responses of the gut to the anaphylactic reaction is also depressed, but the reactivity to the antigen returns earlier than that to histamine or to S.R.S. If the antigen is added to the bath during the period of depressed excitability, the gut is specifically desensitised to it and no longer reacts when its reactivity to S.R.S. and histamine is again normal. Within the first 2 or 3 minutes after lysocithin the gut is insensitive to large doses of histamine and gives only a feeble response even to a large dose of specific antigen. Later the gut may fail to respond to 0·1–0·2 μg. histamine or to 1–2 mg. S.R.S. and yet give an excellent response to a dose of antigen only a little above the threshold. This latter state of affairs is illustrated in figs. 1 and 2. The effect of three doses of 4 mg. of lysocithin on the isolated jejunum from a sensitised guinea-pig is seen in fig. 1, IV. In this experiment the phase of depressed excitability lasted for 8 to 10 minutes, but during the latter part of this time 2 μg. egg albumin caused a response which was somewhat less in extent and had a slightly longer latency (20 seconds) than that given by the control piece of intestine (fig. 1, I). In the experiment of fig. 2 a smaller dose (0·5 mg.) of a strong preparation of lysocithin was used, which gave a period of depressed sensitivity to histamine and S.R.S. lasting from 13 to 16 minutes. In fig. 2, II and III, greater impairment of the anaphylactic response was observed. The intestine in III during the depression phase gave a small response with a latency of 80 seconds to a threshold dose (4 μg.) of egg albumin, while that in II gave a good response with a latency of 35 seconds to 60 μg. egg albumin.

The effect of the antigen in the early part of the period of depressed excitability is seen in fig. 3, which shows the response of three pieces

tion, no response could be obtained to 1 μg. egg albumin, the response to 0·5 μg. histamine being trivial. In III, 6 mg. S.R.S. gave a contraction with a latency of 2 seconds approximately equal in height to the anaphylactic response to 1 μg. egg albumin (I). After irradiation in the presence of hæmatoporphyrin there was no response to 1 μg. egg albumin, but 0·5 μg. histamine still caused a slight response. Partial desensitisation by the first dose of S.R.S. may have contributed to the failure of response to 6 mg. S.R.S. after irradiation.

(v) *The Anaphylactic Response after Treatment with Histaminase (Torantil).*—Since the antigen-antibody reaction is associated with the

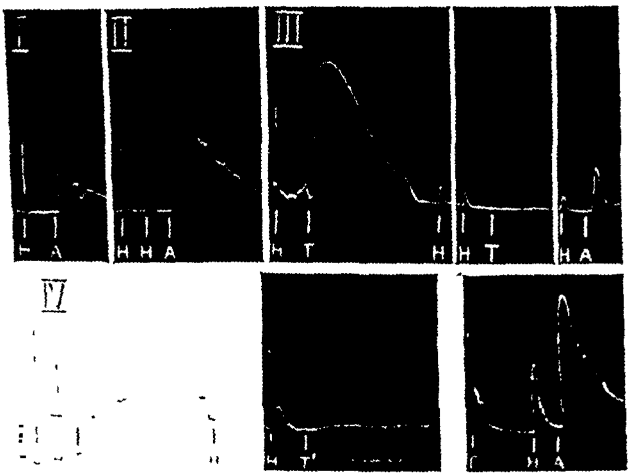

FIG. 6.—Responses of 4 pieces of jejunum from a sensitised guinea-pig. At A, 4 μg. egg albumin; at H, 0·1 μg. histamine; at C, 0·06 μg. acetylcholine; at T, 0·5 unit, and at T', 0·05 unit Torantil. The intervals in the record of III are of 3 and 13 minutes, those in IV of 160 and 20 minutes respectively. Time in minutes. Details in text.

liberation of histamine, we have studied the influence of histaminase on the anaphylactic response of the gut. When a piece of jejunum from a guinea-pig sensitised to egg albumin is left for several hours in Tyrode solution containing histaminase, the response to the anaphylactic antigen is not thereby impaired. Fig. 6, I and II, shows the responses to 4 μg. egg albumin (at A) of two pieces of jejunum from a guinea-pig 42 days after its sensitising injection. In I the jejunum was untreated and in II it had been incubated with histaminase (1 unit of Torantil in 20 c.c. Tyrode solution) for 7 hours at 21° C. The gut in I responded with a latency of 15 seconds and that in II with a latency of 18 seconds, the anaphylactic contraction in II being stronger than in I. A solution of histamine, 1: 2 millions, incubated with the same concentration of Torantil for the same time and at the same temperature had lost 90 p.c. of its activity.

Torantil in doses of 0·2 unit and upwards causes strong contraction

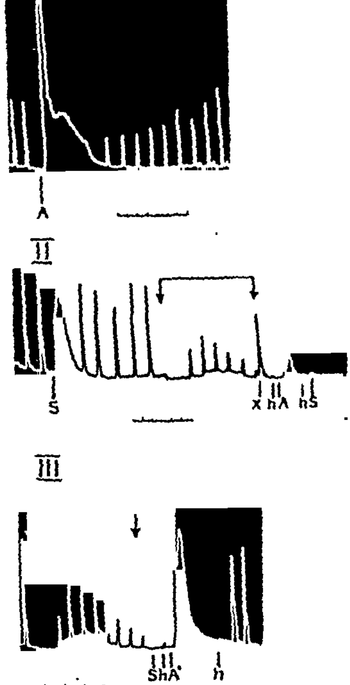

FIG. 4.—Responses of 3 pieces of jejunum from a sensitised guinea-pig. At A, 4 μg., and at A′, 40 μg. egg albumin; and at S, 2 mg. S.R.S. X is a spontaneous emptying contraction. The unlettered contractions and those lettered *h* are to 0·1 μg. histamine except the last two contractions in III, which are to 4 μg. and 6 μg. histamine. The arrows indicate the period of irradiation at the beginning of which 1 mg. hæmatoporphyrin was in contact with the gut for 1 minute. Time in minutes. Details in text.

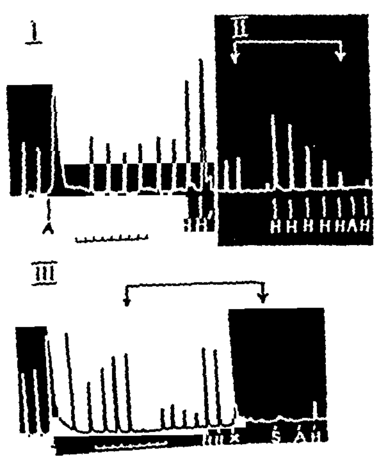

FIG. 5.—Responses of 3 pieces of jejunum from a sensitised guinea-pig. At A, 1 μg. egg albumin; at S, 6 mg. S.R.S.; at H, 0·5 μg., and at H′, 1 μg. histamine. The unlettered contractions are to 0·1 μg. histamine. X marks an emptying contraction after 0·5 μg. histamine. The arrows indicate irradiation as in fig. 4. Time in minutes. Details in text.

that CO_2 diminishes the excitability of the gut. Three to five minutes after the stream of oxygen passing through the bath was replaced by a mixture of 80 p.c. CO_2 and 20 p.c. O_2 the response to 0·1 μg. of acetylcholine was reduced, but that to 0·1–0·5 μg. of histamine, to 1–4 mg. of S.R.S., to 100 times the minimum effective dose of the anaphylactic antigen or to a strongly active dose of cobra venom (10 to 100 μg.) was abolished. When pure oxygen was once more bubbled through the bath the response to histamine became normal

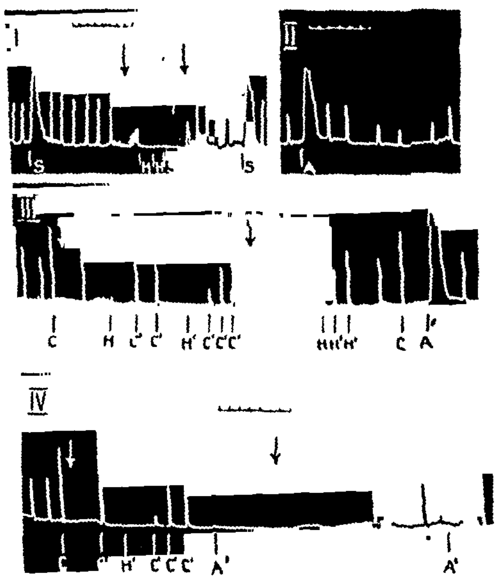

FIG. 7.—Responses of 4 pieces of jejunum, I from a normal guinea-pig, II, III, and IV from a guinea-pig sensitised to egg albumin 28 days previously. Between the arrows in I, III, and IV, CO_2 80 p.c., and O_2 20 p.c. bubbled through the bath. At S, 1 mg. S.R.S.; at A, 1 μg., and at A′, 100 μg. egg albumin. At C, 0·05 μg., and at C′, 0·1 μg. acetylcholine. At H, 0·1 μg., and at H′, 0·2 μg. histamine. The unlettered contractions are to 0·1 μg. histamine. Time in minutes. Details in text.

again after 4 to 10 minutes, and if the anaphylactic antigen or cobra venom had not been given previously they produced their normal contractions. In fig. 7, I, a piece of normal jejunum is shown to have lost its excitability to 0·1 μg. of histamine and to 1 mg. S.R.S. during treatment with 80 p.c. CO_2. The effect of CO_2 on the anaphylactic response is shown in fig. 7, II, III, and IV, at A and A′ on three pieces of jejunum from a sensitised guinea-pig. The response in II (at A) to 1 μg. egg albumin had a latency of 18 seconds and that in III a latency of 20 seconds. The response in III was obtained after treatment with 80 p.c. CO_2 for 17½ minutes and a suitable period for recovery of excitability to histamine. It will be seen that the previous treatment with CO_2 had not impaired the anaphylactic response. In IV the gut

of the jejunum and great diminution of its excitability as indicated by its responses to histamine and acetylcholine. It is, however, possible to administer sub-threshold doses of Torantil to the jejunum and leave them in contact with the muscle for prolonged periods without impairing the response of the gut to histamine or to acetylcholine. In fig. 6, IV, during a total period of 3 hours and 30 minutes, eleven successive doses of 0·05 unit of Torantil each acting for 8 to 15 minutes were added to the bath before testing with egg albumin. The effects of the first and eleventh doses of Torantil are figured. After this treatment the response to histamine (at H), to acetylcholine (at C), and to 4 μg. egg albumin (at A, latency 20 seconds) were unimpaired.

After a single large dose of Torantil the responses of the muscle to histamine, to a second dose of Torantil, and to the antigen are greatly diminished. In fig. 6, III, the addition of a first dose of 0·5 unit of Torantil to the bath (at T) caused a rapid contraction after a latent period of 20 seconds. The Tyrode solution in the bath was changed after a minute and the gut slowly relaxed. The jejunum now failed to react to a second dose of Torantil (at T) and gave only minute responses to 0·2 μg. of histamine (at H) and a much-reduced response to 4 μg. of egg albumin (at A), although the latter had a latency of only 25 seconds. Under similar conditions the response of the jejunum of the normal guinea-pig to cobra venom was apparently unaffected. In one experiment in which Torantil had abolished the excitability of the gut to 0·2 μg. histamine, 10 μg. cobra venom produced a large sustained contraction with a latency of 12 seconds.

(vi) *The Effect of CO_2 on the Anaphylactic Response.*—Garan [1937] has shown that bubbling CO_2 through a solution containing histamine renders it inactive when tested on the guinea-pig's jejunum and has attributed this inactivation to the formation of a readily dissociable compound of histamine with CO_2. We have been able to confirm this observation, but our results suggest a different explanation. When CO_2 is bubbled through a solution of histamine (1 : 2 millions) for a few minutes, the response of the gut to 0·2 c.c. of the solution is much less than that to 0·2 c.c. of the untreated solution, but a similar depression of the response to this dose of histamine is obtained, when 0·2–0·4 c.c. of Tyrode solution through which CO_2 has been bubbled is added to the bath immediately before the histamine is tested. S.R.S. exhibits similar behaviour though the effect of CO_2 is not so striking, possibly because the slower reaction to S.R.S. permits time for the removal of CO_2 in the bath by the oxygen bubbling through it. These results suggest that the inactivating action of CO_2 is attributable to its depressant action on the gut, in which case we are provided with another method for producing transient depression of the excitability of the sensitive jejunum.

The results obtained afford further evidence in support of the view

dose selected only suffices to produce a submaximal contraction in the untreated gut. This is illustrated in fig. 9, which shows the responses of three pieces of jejunum from a guinea-pig sensitised 91 days earlier by the subcutaneous injection of 15 mg. of egg albumin. The sensitivity of the gut to egg albumin is shown by the responses in the upper two panels (at A and A′) to 5 and 0·05 μg. respectively of the antigen. These responses had latencies of 9 and 20 seconds. The lower panel shows the responses of a third piece of jejunum to 1 mg. S.R.S. (at S)

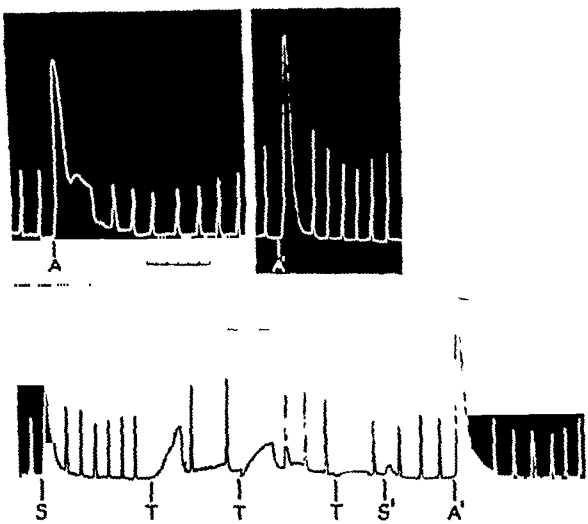

Fig. 9.—Responses of 3 pieces of jejunum from a sensitised guinea-pig. At A, 5 μg., and at A′, 0·5 μg. egg albumin; at S, 1 mg., and at S′, 4 mg. S.R.S.; at T, 4 mg. *Cl. welchii* type A toxin. The unlettered responses are to 0·06 μg. histamine. Time in minutes. Details in text.

with a latency of 7 seconds. Two doses of 4 mg. of toxin (at T) each left in contact with the gut for 3 minutes gave responses with latencies of 35 and 20 seconds, and a third dose gave no response. The gut now gave only a trivial response to 4 mg. of S.R.S. (at S′), but reacted to 0·05 g. of egg albumin (at A′) with a latency of 21 seconds.

B. *The Isolated Uterus of the Virgin Guinea-Pig.*

Lysocithin.—Lysocithin causes a strong sustained contraction of the virgin uterus followed by a period of decreased excitability to histamine [Feldberg, Holden, and Kellaway, 1938]. After repeated doses the muscle ceases to respond to lysocithin, its response to histamine is reduced but not abolished, and it can no longer respond to S.R.S. In this condition the anaphylactic response is reduced to the same degree as that to histamine. These statements are illustrated in

failed to respond to 100 μg. egg albumin after treatment for 10 minutes with CO_2. When the response to histamine was again normal after changing back to O_2, the gut was found to have been desensitised to the antigen failing to respond to a second dose of 100 μg. of egg albumin.

Since the reactivity of the gut to histamine and to acetylcholine is influenced in different degrees by CO_2 [Garan, 1937], we tried to obtain a similar differentiation for histamine, S.R.S., and antigen by using CO_2 in lower concentrations, but we were not able to achieve this. A mixture of 30 p.c. CO_2, 50 p.c. N_2, and 20 p.c. O_2 gave variable results depending on the period of exposure to the CO_2 mixture. After 2–3 minutes the gut invariably failed to respond to 0·2 μg.

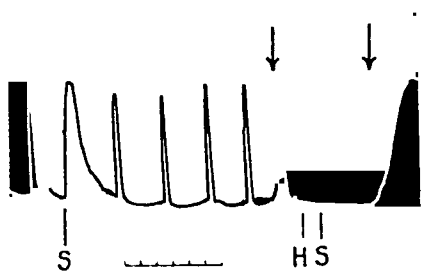

Fig. 8.—Responses of jejunum of a guinea-pig. At S, 1 mg. S.R.S.; at H, 0·2 μg. histamine. The unlettered responses are to 0·1 μg. histamine. Between the arrows 30 p.c. CO_2, 50 p.c. N_2, and 20 p.c. O_2 bubbled through the bath. Time in minutes. Details in text.

histamine, to 2 mg. S.R.S., and to about twice the threshold dose (1 μg.) of antigen, but gave a large response to 100 μg. egg albumin. After 10 minutes exposure even the response to this large dose of antigen failed. When 1 or 2 minutes after washing out the antigen or S.R.S. the stream of CO_2 mixture was replaced by oxygen, a prolonged contraction often followed which was probably a delayed response to the antigen or to S.R.S. This is illustrated for 1 mg. of S.R.S. in fig. 8. A similar phenomenon was observed after the administration of 80 p.c. CO_2, when cobra venom (100 μg.) had been given. When oxygen was again bubbled through the bath a strong contraction occurred.

(vii) *The Anaphylactic Response after Treatment with the Toxin of Cl. welchii A.*—The repeated administration of this toxin to the jejunum greatly diminishes its reactivity to S.R.S., but does not impair its response to histamine [Kellaway and Trethewie [1]]. It seemed worth while to study its effect upon the anaphylactic response. A piece of jejunum from a sensitised guinea-pig after treatment with toxin gives an unimpaired response to the anaphylactic antigen even when the

[1] Unpublished work.

65, and 120 seconds. The responses to the sixth and seventh doses were trivial, and there was no response to the eighth dose of lysocithin. The uterus now failed to react to its first dose of 4 mg. S.R.S. and gave a maximal contraction with a latency of 20 seconds to 1 mg. egg albumin. This response was equivalent to that given by 5 μg. histamine, which was not appreciably less in extent than that obtained before treatment with lysocithin.

S.R.S.—The behaviour of the uterus to repeated doses of S.R.S. is peculiar but has been observed regularly in a number of experiments. The earlier responses to S.R.S. have increasing latencies and show progressive decrease in extent, but after four or five responses the contractions again become stronger and their latencies are not further increased. At this stage the natural rhythm and the response of the muscle to histamine are increased. The uterus is not desensitised to the specific antigen by treatment with S.R.S. however prolonged. For example, one horn of the uterus of a guinea-pig 34 days after a sensitising injection gave a submaximal response with a latency of 12 seconds to 4 μg. egg albumin. The other horn after treatment with five doses of 4 mg. and three doses of 8 mg. S.R.S., administered at intervals of half an hour and left in contact with the muscle for 6 minutes, gave a maximal response with a latency of 15 seconds to 20 μg. egg albumin.

Carbon Dioxide.—Bubbling a mixture of 80 p.c. CO_2 and 20 p.c. oxygen through the bath caused reversible depression of the excitability of the muscle. The sensitised uterus now failed to respond to 8 mg. S.R.S., to 2 mg. lysocithin, to 10 μg. histamine, to 0·2 unit of pitressin, to 1 μg. acetylcholine, and to ten times the effective dose of anaphylactic antigen. When oxygen was again bubbled through the bath the excitability of the muscle shortly returned to normal. If it had not been tested with the antigen during the phase of depressed excitability the uterus was still fully sensitive to the antigen, but if it had been tested with antigen it could no longer give an anaphylactic response, having been desensitised without reaction.

C. *The Perfused Lung.*

(i) *Liberation of a Slow-reacting Muscle-stimulant Substance from the Perfused Lung during Anaphylactic Shock.*—Bartosch, Feldberg, and Nagel [1932] have shown that the injection of the anaphylactic antigen into the perfused lung of the sensitised guinea-pig caused the liberation of histamine. Our experiments show in addition that a substance which in its action on the isolated jejunum of the guinea-pig resembles that formed by the action of snake venoms on egg yolk or perfused tissues [Feldberg, Holden, and Kellaway, 1938] appears in the outflowing perfusate from the lung after the anaphylactic response.

fig. 10, which shows the responses of the two horns of the uterus of a guinea-pig 24 days after a sensitising injection of egg albumin. The left horn gave a moderate response with a latency of 20 seconds to 2 mg. of S.R.S. and a submaximal response with a latency of 25 seconds to 40 μg. egg albumin. The uterus was now desensitised to this dose of antigen. The response of the right horn to 3 μg. histamine was maximal, and 2 mg. of S.R.S. gave a response similar to that of the other horn. Four doses of lysocithin (5 mg.) gave progressively diminishing contractions, the first three with latencies

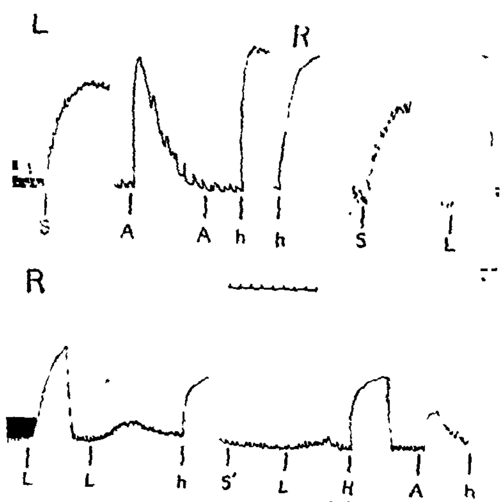

FIG. 10.—Responses of the 2 horns (L and R) of the uterus of a sensitised guinea-pig. At S, 2 mg., and at S′, 4 mg. of S.R.S.; at A, 40 μg. egg albumin; at L, 5 mg. lysocithin; at *h*, 3 μg., and at H, 12 μg. histamine. Time in minutes. Details in text.

of 7, 30, and 50 seconds. The muscle now gave no reaction to 4 mg. of S.R.S. During this treatment the uterus, though still able to contract to histamine, gave responses of diminished extent, that to 12 μg. being only half as great as the response obtained at first with 3 μg. With 40 μg. egg albumin, after a latent period of 30 seconds. it gave a contraction of about the same size as that caused by 3 μg. histamine. In some experiments the diminution of the response to histamine and to the anaphylactic antigen appeared to be related to the fact that the muscle gradually became contracted during treatment with lysocithin. Sometimes the responses to histamine and to the anaphylactic antigen were not significantly reduced by lysocithin. This was the case in an experiment in which eight successive doses of 5 mg. of lysocithin were left in contact, each for 5 minutes, with the uterus of a guinea-pig 23 days after the sensitising injection. The latencies of the first five responses to lysocithin were 20, 20, 40,

fourth sample (collected in $4\frac{1}{2}$ minutes) contained only a trace, and the fifth and sixth samples were free from detectable amounts of histamine. There was no S.R.S. in the first sample after injection, but it was present in moderate amounts in the second and third samples and in diminishing amounts in the fourth, fifth, and sixth samples. When the lung was cut down after the experiment, 1·3 c.c. of fluid drained off during 10 minutes. This fluid contained histamine in a concentration of 1: 7 millions and a small amount of S.R.S. The lung, which was œdematous, now contained only 10 μg. histamine per gram. In fig. 11 the contractions at A, B, D, E, F, and G are to 1 c.c. of the six successive samples collected after the injection, and that at H to 1 c.c. of drainage fluid. The samples were left in contact with the gut for 10 seconds before washing out. The steep contractions at A, B, and D, having no latency, are attributable to histamine, whereas the presence of S.R.S. is shown only by delay in relaxation. The contraction of the fifth sample (at F) had a latency of 5 seconds and must be attributed wholly to S.R.S. The contraction of the fourth sample (at E) started immediately, but only a small portion of it was due to histamine. This was evident when the fourth and fifth samples were retested upon the following day, having been kept overnight in the refrigerator. By this time the trace of histamine in the fourth sample had disappeared, and the contractions produced by both samples had a latency of 5 seconds like that at F in fig. 11. In fig. 12 an experiment is illustrated in which the output of histamine was small, but that of S.R.S. substantial. The lungs of a guinea-pig weighing 425 g. 46 days after the sensitising injection were perfused at a rate of 3 c.c. per minute. At B the effect of 1 c.c. of a sample collected a few minutes before the injection of 20 mg. of egg albumin in 0·2 c.c. was tested, and at C that of 4 mg. albumin in 1 c.c. of this sample of perfusate. The responses were trivial. The shock after the injection was slow in onset and only complete in 4 minutes. The first three samples after the injection contained 1 μg. histamine, which was present in concentrations of 1: 25, 1: 12, and 1: 17 millions. The fourth and fifth samples, which were collected at a slower rate of flow (1·5 c.c. per minute), contained no detectable amount of histamine. S.R.S. was present in traces only in the first and second samples and in substantial amounts in the following three samples after injection. The responses to 1 c.c. of the fourth and fifth samples are seen at A and D in fig. 12, the latencies being 7 and 5 seconds respectively.

(ii) *Effect of Antigen on the S.R.S. Content of Lung Extracts.*—The appearance of S.R.S. in the venous outflow from a perfused lung when anaphylactic shock has been produced may have been due to formation of this substance or to its liberation from pre-existing stores in the tissue. In order to decide this question, lobes of the lungs before and after the antigen had acted upon them were extracted and assayed for

This substance may be identical with that formed by the action of snake venom, though we have no proof that this is the case. For convenience, however, we shall refer to it as S.R.S. We found that there was much variation in the amounts of histamine and S.R.S. appearing in the perfusate. The maximum output of histamine preceded that of S.R.S., and the highest concentrations observed ranged from 1 in 1 million to 1 in 20 millions. The output of S.R.S. bore no relation to the amount of histamine liberated.

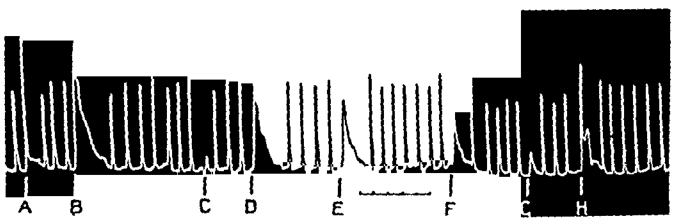

Fig. 11.—Responses of jejunum of a guinea-pig to perfusate from the isolated lungs of a sensitised guinea-pig. The unlettered contractions are to 0·1 μg. histamine. Time in minutes. Details in text.

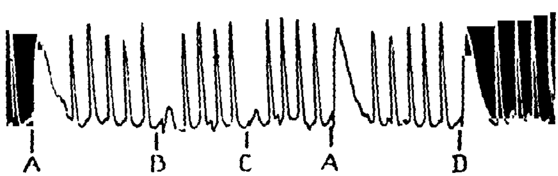

Fig. 12.—Responses of jejunum of a guinea-pig to 1 c.c. of perfusates from the isolated perfused lung of a sensitised guinea-pig. At B, a sample before, at A and D, samples collected after the injection of antigen; and at C, 4 mg. egg albumin in 1 c.c. of B. Time in minutes. Details in text.

Our main results are illustrated in figs. 11 and 12. Fig. 11 shows tests of perfusate from the lung of a guinea-pig weighing 395 g. 50 days after the sensitising injection. The lung, a piece of which before the injection of antigen was found to contain 25 μg. histamine per gram, was perfused at a rate of 1·5 c.c. per minute, and the perfusate, before the injection of antigen, had no appreciable action on the isolated gut of the guinea-pig in doses of 1 c.c. (at C). After the injection of 20 mg. egg albumin in 0·2 c.c. shock was observed in 15 seconds and was complete in 40 seconds. The first three samples collected during successive periods of $3\frac{1}{2}$, 4, and 5 minutes after the injection contained a total of 3 μg. histamine which was present in concentrations of 1: 3·5, 1: 7, and 1: 10 millions. The

appearance of histamine and S.R.S. in the perfusate. Perfusion was stopped 12 minutes after the injection, when the activity of the perfusate had again greatly diminished. The lung was cut down, weighed, and extracted after some fluid had drained off. The weight of the combined lobes was 1·7 g., but the amount of tissue in the extract was based on the weight of 0·68 g. of the non-œdematous tissue. The effect of egg albumin in increasing the S.R.S. yield of saline extracts was only obtained in sensitised guinea-pigs; in normal animals the egg albumin had no effect of this kind whether injected into the perfused lung or

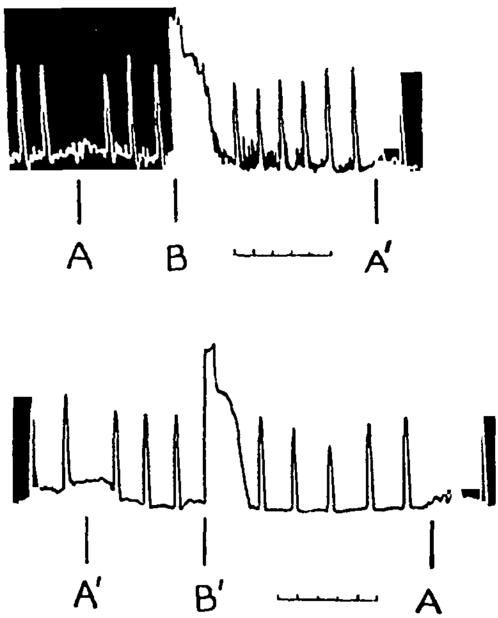

Fig. 14.—Responses of the isolated jejunum of a guinea-pig to sediment and supernatant fluid of an extract of desiccated dog's lung. A, boiled, and A′, unboiled sediment; B, boiled, and B′, unboiled supernatant fluid equivalent to 5 mg. tissue. The unlettered contractions are to 0·1 μg. histamine. Time in minutes. Details in text.

allowed to act upon the ground-up organ *in vitro*. It seemed therefore likely that S.R.S. was formed by the action of antigen upon sensitised tissue.

On the other hand, simple extraction may not have removed all the available S.R.S. in normal tissue, and the increase after anaphylaxis may have resulted from the easier extraction of S.R.S. from cells injured by the antigen-antibody reaction. We therefore used extracts of desiccated tissue [Kellaway and Trethewie, 1939], which always contain more S.R.S. than simple saline extracts. It is possible that

their S.R.S. content. Simple saline extraction of the fresh tissue gave results different from those obtained on extracting the desiccated tissue. The effect of antigen was to increase the yield of S.R.S. in the simple saline extracts, but not that in the extracts of desiccated tissue. An experiment with simple saline extraction is illustrated in fig. 13 in which A shows the effect of extract of untreated right lung, B that from the left lung after the anaphylactic shock had been produced in the perfused organ, and C that from the right lung the extract of which had been incubated with egg albumin. It will be seen that the lung

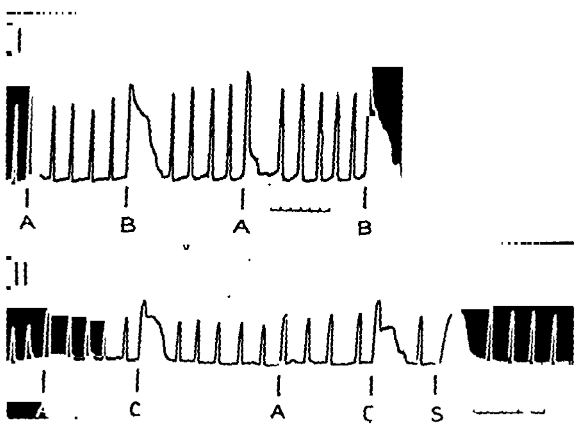

Fig. 13.—Responses of jejunum of a guinea-pig. At A, B, and C to extracts of perfused lung from a sensitised guinea-pig; at S, 1 mg. S.R.S. (latency 12 seconds). The unlettered responses are to 0·1 μg. histamine. Extracts left in contact with gut 15 seconds. Time in minutes. Details in text.

yields more S.R.S. when the anaphylactic shock has been produced or the ground-up tissue incubated *in vitro* with the antigen than when no antigen has been allowed to act on the tissue. The extracts were obtained from a guinea-pig weighing 268 g. 32 days after the sensitising injection and made up so that 0·2 c.c. contained 5 mg. lung tissue, the amount used for the tests. Both lungs had been perfused, the right lung being removed after some minutes. It weighed 0·68 g. Part of it was ground up in saline solution, boiled, and assayed at A. It had a histamine equivalent of 27 μg. per gram. The other part, 0·28 g., was ground up in saline solution and incubated for 15 minutes at 37° C. with 20 mg. egg albumin before it was boiled and tested at C. Incubation without the addition of egg albumin did not change the effect of the extract. The contractions at A in II were obtained from extracts incubated without egg albumin. The left lung and the intermediate lobe, the combined weight of which equals that of the right lung, were perfused at a rate of 2 c.c. per minute. An injection of 20 mg. of albumin produced strong broncho-constriction and the

D. *The Effect of Antigen on the S.R.S. Content of Extracts from the Perfused Jejunum, Uterus, and Liver of Sensitised Guinea-Pigs.*

(i) *Jejunum.*—Extracts from adjacent portions of the perfused jejunum, prepared by grinding the fresh tissue with silica and saline, contained about the same amount of histamine and of a slow-reacting substance which may be identical with the substance formed by the

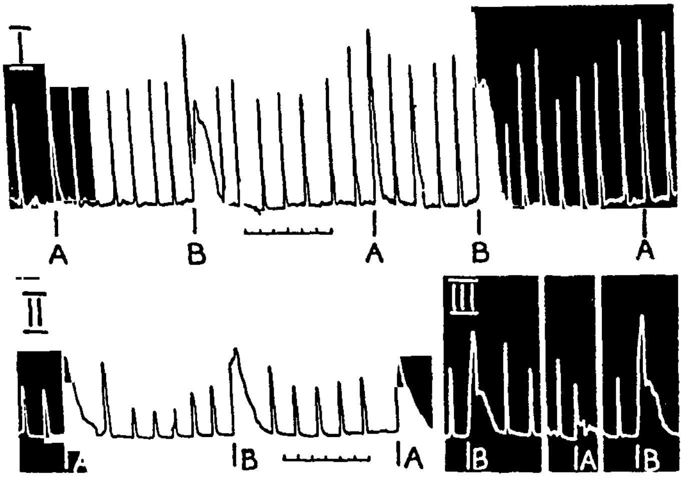

FIG. 16.—Records of isolated jejunum from 3 guinea-pigs. Jejunum in III desensitised to cobra venom. Unlettered responses are to 0·1 μg. histamine. Extracts left in contact with gut for 10 seconds in I, for 15 seconds in II, for 30 seconds in III. Time in minutes.

(I) Effects of saline extracts equivalent to 2·5 mg. tissue from perfused jejunum of 190 g. guinea-pig sensitised 21 days previously. A before, B after arterial injection of 35 mg. egg albumin.

(II) Effects of desiccated saline extracts, equivalent to 10 mg. tissue, from perfused jejunum of 280 g. guinea-pig sensitised 32 days previously. A before, B after incubation with 66 mg. egg albumin per gram tissue.

(III) Effects of desiccated saline extracts, equivalent to 5 mg. tissue, from jejunum of normal guinea-pig. A before, B after incubation with 1 mg. cobra venom per gram tissue.

action of venoms on tissues and will therefore be referred to as S.R. When one piece of jejunum was extracted before and another aft… an arterial injection of egg albumin into the perfused gut of a sensitised guinea-pig, this method of extraction yielded less histamine but more S.R.S. from the injected piece than from the control one. but when the extracts were desiccated and re-extracted they failed to reveal any difference. Similar results were obtained on the S.R.S. content when egg albumin was allowed to act *in vitro* upon saline extracts of a piece of perfused jejunum. The amount of S.R.S. increased in the extracts incubated with albumin, but the difference disappeared when the extracts were desiccated and re-extracted. When extracts from the

some S.R.S. is formed during the process of desiccation, but mor likely that the increased yield results from better extraction. Indeed the extraction appears to be complete, since after centrifugation of th re-extracted desiccated tissue the total activity of histamine as well a of S.R.S. was found in the supernatant fluid, whereas none could b detected in the residue when it was ground up again and tested, boile or unboiled. This is illustrated in fig. 14 for an extract made from th

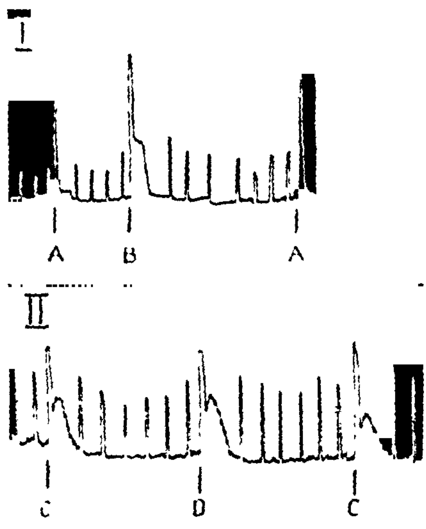

Fig. 15.—Responses of 2 pieces of guinea-pig's jejunum to extracts of the perfused lung from a guinea-pig sensitised 41 days previously. In I simple saline extracts equivalent to 12·5 mg. and in II desiccated extracts equivalent to 5 mg. tissue; A and C, control extracts; B and D, extracts of lung incubated with egg albumin. The unlettered responses are to 0·1 μg. histamine. Time in minutes. Details in text.

normal lung of the dog. In five experiments on the perfused lungs sensitised guinea-pigs in which the tissue was desiccated before i final extraction, incubation of the ground-up tissue with egg album did not increase the yield of S.R.S. In fig. 15 the different resul obtained with the two methods of extraction are illustrated. In tl experiment the simple saline extract had to be tested in a dose equivale to 12·5 mg. of tissue. There was an increase in histamine and S.R. after incubation with egg albumin (at B). After desiccation (in I extract equivalent to 5 mg. of tissue only was needed for the test an the incubation with egg albumin before desiccation had caused i increase in the yield of S.R.S. or of histamine. It appears like therefore that the antigen-antibody reaction causes liberation but n formation of S.R.S. in the lung.

D. *The Effect of Antigen on the S.R.S. Content of Extracts from the Perfused Jejunum, Uterus, and Liver of Sensitised Guinea-Pigs.*

(i) *Jejunum.*—Extracts from adjacent portions of the perfused jejunum, prepared by grinding the fresh tissue with silica and saline, contained about the same amount of histamine and of a slow-reacting substance which may be identical with the substance formed by the

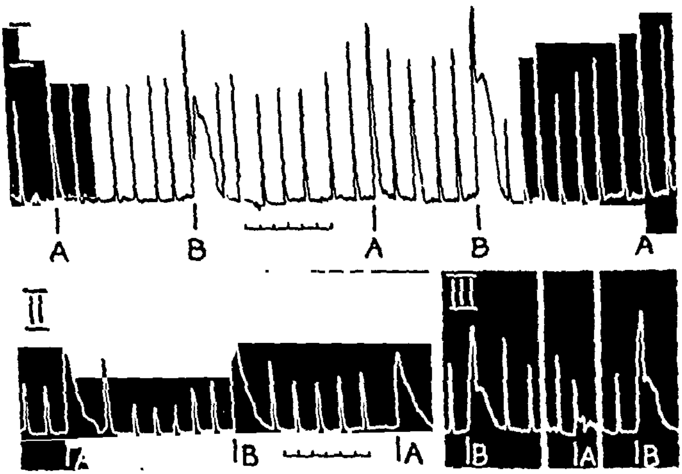

FIG. 16.—Records of isolated jejunum from 3 guinea-pigs. Jejunum in III desensitised to cobra venom. Unlettered responses are to 0·1 μg. histamine. Extracts left in contact with gut for 10 seconds in I, for 15 seconds in II, for 30 seconds in III. Time in minutes.

(I) Effects of saline extracts equivalent to 2·5 mg. tissue from perfused jejunum of 190 g. guinea-pig sensitised 21 days previously. A before, B after arterial injection of 35 mg. egg albumin.

(II) Effects of desiccated saline extracts, equivalent to 10 mg. tissue, from perfused jejunum of 280 g. guinea-pig sensitised 32 days previously. A before, B after incubation with 66 mg. egg albumin per gram tissue.

(III) Effects of desiccated saline extracts, equivalent to 5 mg. tissue, from jejunum of normal guinea-pig. A before, B after incubation with 1 mg. cobra venom per gram tissue.

action of venoms on tissues and will therefore be referred to as S.R.S. When one piece of jejunum was extracted before and another after an arterial injection of egg albumin into the perfused gut of a sensitised guinea-pig, this method of extraction yielded less histamine but more S.R.S. from the injected piece than from the control one. but when the extracts were desiccated and re-extracted they failed to reveal any difference. Similar results were obtained on the S.R.S. content when egg albumin was allowed to act *in vitro* upon saline extracts of a piece of perfused jejunum. The amount of S.R.S. increased in the extracts incubated with albumin, but the difference disappeared when the extracts were desiccated and re-extracted. When extracts from the

jejunum were incubated with cobra venom there was an increase in the yield of S.R.S. not only in simple saline extracts but also in the desiccated saline extracts. These results are illustrated in fig. 16 by three experiments, the details of which are given in the legend of the figure.

(ii) *Uterus and Liver.*—When saline extracts of the uterus or liver from sensitised animals were tested on the isolated jejunum in doses equivalent to a few mg. of tissue they gave only a trivial S.R.S.-like response or none at all. In contrast to the results obtained with extracts of the intestine and lung, incubation with egg albumin did not influence the activity of such extracts.

Discussion.

The similarity of the anaphylactic contraction of the jejunum of the guinea-pig to that caused by venom provides some basis for the view that this contraction may depend at least in part on the formation or liberation of a substance like the S.R.S. formed by snake venoms. The study of this response under a variety of conditions of decreased reactivity of the muscle failed to give a decisive answer to the question as to whether the anaphylactic contraction results from the liberation of histamine or from the liberation or formation of S.R.S. In comparing the responses of the muscle to histamine, to S.R.S., and to the antigen under these conditions, we have not only to take into account the likelihood that these various kinds of depressed reactivity have resulted from wholly different changes in the muscle, but also the possibility that the modes of action of the three stimuli are different. The action of histamine on the muscle is direct and immediate, and that of the antigen indirect and delayed, probably therefore resulting from the liberation or production of active substances. S.R.S. causes a delayed response after a definite though short latent period, and it is not inconceivable that this also is determined indirectly by liberation of a stimulating principle.

We have summarised the results of the experiments on the jejunum of the guinea-pig in Table I. It will be seen that most of the conditions of decreased reactivity reduced the responses of the gut to antigen, to histamine, and to S.R.S. to about the same extent, or that the differences observed were too slight to allow any conclusions to be drawn from them. There were, however, three exceptions. In the late stage of lysocithin poisoning, when recovery was taking place, the anaphylactic response was but little affected though the responses to histamine and to S.R.S. were greatly reduced. This result does not support either the contention that histamine on the one hand or that S.R.S. on the other is the chief contracting principle in anaphylaxis. It is, however, not unlikely that if active substances are liberated within

TABLE I.

Treatment.	Responses of the jejunum to		
	S.R.S.	*Histamine.*	*Antigen.*
S.R.S.	Almost abolished	Unimpaired	Slightly reduced
Lysocithin (early stage)	Abolished	Almost abolished	Almost abolished
Lysocithin (late stage) .	Greatly reduced	Greatly reduced	Slightly reduced
Photodynamic action .	Greatly reduced	Greatly reduced	Greatly reduced
Torantil	..	Greatly reduced	Greatly reduced
CO_2 high concentration	Abolished	Abolished	Abolished
CO_2 low concentration .	*Reduced*	*Reduced*	*Reduced*
Toxin of *Cl. welchii* A .	Greatly reduced	Unimpaired	Unimpaired

a muscle they are more effective stimuli than when applied to its outer surface. In the other two exceptional cases after repeated administration of S.R.S. and after treatment with the toxin of *Cl. welchii* type A, the response to S.R.S. was greatly reduced, whereas those to histamine and to the anaphylactic antigen were unimpaired or only slightly affected. A similar dissociation of the responses was obtained by treating the uterus of the guinea-pig with repeated doses of lysocithin. These results favour the hypothesis that histamine and not S.R.S. is responsible for the anaphylactic contraction in both muscles, and differentiate between the anaphylactic response of the gut and that due to cobra venom because after treatment with the toxin of *Cl. welchii* type A the gut fails to respond to an initial dose of venom. On the other hand, it has been shown that when the uterus is poisoned by large doses of histamine it reacts to further doses by relaxation, whereas S.R.S. and the anaphylactic antigen still cause contraction although to a somewhat reduced extent. We are thus unable from a comparison of the contractions of these varieties of plain muscle under different conditions of reduced reactivity absolutely to exclude either histamine or S.R.S. as stimulant principles participating in the anaphylactic response. There remains the possibility that both substances are involved, an assumption which is supported by the experiments now to be discussed.

The liberation of histamine during the antigen-antibody reaction has been demonstrated for various organs and under different experimental conditions. In our experiments also histamine appeared in the perfusate from sensitised lungs after the arterial injection of the antigen, but in addition there appeared a substance (or substances) with an action on the isolated gut like S.R.S. formed by venoms. Though we describe this as S.R.S. we have not established its identity with the substance formed by venom. We have, however, attempted

to find out whether it is liberated or formed. The increase of S.R.S. in fresh extracts of the perfused lung and intestine after arterial injections of the antigen or incubation with it suggested formation of S.R.S., and it thus appeared that in sensitised animals the antigen exerted an enzymic function similar to that of venoms and that S.R.S. might be regarded as a cleavage product derived from the lipins during such reaction. Further experiments, however, with dried tissue extracts, which gave a greater yield of S.R.S. from normal tissues than did fresh extracts, revealed no increase in S.R.S. content when the antigen had been allowed to act on the tissue. It is therefore more likely that the yield of S.R.S. obtained by saline extraction of fresh tissue is incomplete and that the increase observed after the action of antigen must be explained not by formation of S.R.S. but by its liberation from the tissue, where it may be normally held in an inactive form within the lipo-protein structure of the protoplasm or loosely bound to some other substance. Though we cannot be certain what are the effects of drying upon the constituents of a tissue and whether this in itself may not result in the liberation of S.R.S., it should be noted that, in agreement with the conclusion that S.R.S. is formed by venom, desiccated extracts of tissue incubated with cobra venom show a striking increase in S.R.S. over that present in control extracts.

The amount of S.R.S. detected in extracts as a result of the antigen-antibody reaction was much less than that obtained in the experiments with venoms, and though this quantitative difference might be attributed to our inability to produce reactions with antigen as strong as those produced by venoms, it is more likely that the difference is a qualitative one and is possibly even dependent on an essential difference in nature between the superficially similar slow-reacting substances formed in the case of cobra venom and liberated from the tissue cells in the case of the anaphylactic reaction.

Conclusions.

1. The response of the isolated jejunum of the sensitised guinea-pig to the anaphylactic antigen resembles that produced by venoms and by S.R.S. in its latency, in the time required for relaxation, and in the after-changes in excitability of the muscle.

2. The contractions of the jejunum of the guinea-pig to antigen, to histamine, and to the slow-reacting muscle-stimulant substance (S.R.S.) formed by venoms were compared under various conditions of decreased reactivity of the muscle. These were brought about by administration of S.R.S., lysocithin, histaminase (Torantil), CO_2, the toxin of *Cl. welchii* type A, and by photodynamic action. In most cases the responses to histamine, S.R.S., and antigen were depressed to the same extent. After repeated treatment with S.R.S. the response

to S.R.S. was greatly reduced or abolished, that to histamine was unimpaired, and the response to the antigen was only slightly affected. During recovery from lysocithin poisoning the response to the antigen was only slightly reduced, while those to S.R.S. and histamine were greatly reduced. After treatment with the toxin of *Cl. welchii* type A the response to S.R.S. was greatly reduced, whereas those to histamine and to the antigen were unimpaired. A similar dissociation was observed with the guinea-pig's uterus after lysocithin.

3. The inability of the jejunum of the guinea-pig to respond to histamine when CO_2 has been bubbled through the solution containing it does not result from the formation of a loose but inactive compound of histamine with CO_2, but from an effect of CO_2 on the muscle, causing a decrease in its reactivity to various stimuli.

4. The intra-arterial injection of antigen into the isolated perfused lung of the guinea-pig causes the liberation of a slow-reacting muscle-stimulant substance into the outflowing perfusate.

5. Fresh saline extracts of sensitised lung show an increase in their S.R.S. content after incubation with antigen, but extracts of tissue desiccated after treatment with antigen and subsequently re-extracted show no such increase.

6. Fresh and desiccated extracts of sensitised jejunum incubated with antigen behave like lung extracts, but fresh saline extracts of sensitised uterus and liver similarly treated show no increase in activity.

7. Desiccated as well as fresh extracts of intestine treated with cobra venom show a striking increase in their content of S.R.S.

8. It is concluded that the anaphylactic contraction of smooth muscles is in part due to the liberation of histamine and probably in part also to S.R.S., which is liberated and not formed in the antigen-antibody reaction.

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ABSORPTION FROM THE JOINT CAVITY.[1] By E. W. O. ADKINS and D. V. DAVIES. From the Anatomy School, Cambridge.

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A CLEAR conception of the pathways involved in the removal of true solutions and particulate matter from the joint cavity is essential, either from the point of view of the absorption of normal synovial fluid or of effusions into the joint.

Little attention seems to be paid to the limited literature on this subject by the orthopædic surgeons of the present day. One of the earliest observers was von Mosengeil [1876] who gave an account of the absorption of ink particles from the knee-joints of rabbits. This was followed by Braun [1894] who gave an excellent description of the absorption of colloidal particles from joint cavities. More recent workers have been Fisher [1923] on the absorption of both colloidal and true solutions; Key [1926], whose paper on the removal of colloidal carbon particles followed closely the lines taken by Braun; and, later, Bauer, Short and Bennett [1933] on the absorption of proteins from the knee-joint in dogs.

The diarthrodial joint, characterized by the presence of a cavity containing synovial fluid, presents two tissues which are biologically quite distinct. On the one hand, the hyaline articular cartilage is relatively bloodless, has low power of repair and no visible nerve-endings. On the other hand, the synovial membrane is richly supplied with blood by the circulus vasculosus of Hunter, has high power of proliferation and a rich supply of nerve-endings. The possible routes of absorption are either into the subsynovial tissue spaces, into the capillaries of the circulus vasculosus or into the lymphatic vessels.

The cell count of the synovial fluid is such as to suggest that certain forms of particulate matter may be carried by the cells themselves from the joint cavity to one or more of the three destinations mentioned above. On the other hand, absorption from the joint cavity may be independent of the action of any of the cells in the synovial fluid or membrane. The present paper will deal mainly with the pathways involved in absorption from the joint cavity and, where possible, with the mechanical and physical factors concerned. Little attention will be paid to the tissue reactions.

[1] Contribution to a discussion in the Section of Anatomy and Anthropology at the Annual Meeting of the British Medical Association, Aberdeen, 1939.

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membrane, as no difference in the rate of absorption is observed when these cells are injured by sodium fluoride. Osmosis and diffusion would account for the absorption of true solutions. The diffusion of uroselectan from the knee-joint of the rabbit into the surrounding tissues can be followed radiographically after injection of 1 c.c. of the solution into a recently killed animal. This is seen to occur more particularly through the posterior part of the capsule of the knee by a gradual spread of the radio-opacity in this direction. Similar results have been obtained using sodium iodide solutions and following them radiographically (fig. 1).

A B

FIG. 1.—Radiographs of the left knee-joint of a rabbit immediately (A) and one hour (B) after the injection of 1 c.c. of uroselectan B.

That uroselectan B is mainly absorbed by the blood-stream is indicated by the fact that ligature of the femoral sheath in the femoral triangle does not affect its rate of removal from the joint. This procedure completely occludes the lymphatic pathways of the thigh and leg, while the arterial and venous anastomosis in the lower limb seems adequate to ensure that the normal functions of the tissue are carried out.

These experiments would seem to indicate that lymphatic absorption plays but a minor rôle, if any, in the absorption of true solutions. The injection of 1 c.c. of a 1 p.c. solution of methylene blue into the knee-joint of a rabbit shows that there is some absorption into lymphatics. On killing the animal $1\frac{1}{2}$ hours later, the dye could be seen in the lumbar and thoracic lymph channels. In a similar experiment with the animal under continuous anæsthesia and the skin of the lower limb removed, the diffusion of this dye into the neighbouring tissues could be observed over a considerable period of time.

Absorption of Colloidal Solutions.

Most observers have emphasized that the main pathway for the drainage of colloidal particles from joint cavities is into the lymphatic

Materials and Method.

These experiments have been performed on the knee-joints of normal rabbits. To prevent leakage from the cavity of the knee after injection, the needle has been introduced through the patellar tendon. Injections made through the looser parts of the capsule have resulted in a high percentage of leakages, particularly if a large amount of fluid has been introduced.

Anatomical Considerations.

Two anatomical points seem to be worthy of mention here. The extensor digitorum longus muscle of the rabbit arises by a tendon within the capsule of the knee-joint from the lateral condyle of the femur. The tendon carries with it in passing through the capsule a prolongation of the synovial membrane of the knee-joint in much the same way as does the tendon of the long head of the biceps in the human shoulder. Substances injected into the knee-joints of the rabbit spread along this tendon sheath and then along the muscle sheath, aided by gravity and movement, as far as the ankle-joint. This is seen in almost all the injection experiments on the knee-joints of rabbits. The lymphatics draining this muscle sheath have on more than one occasion been observed draining into the popliteal lymph gland. Little notice seems to have been taken of this spread along the tendon and muscle sheath of the extensor digitorum, and it would appear that the choice of the dog for these experiments by Bauer, Short and Bennett is more satisfactory as it avoids this complication.

The second anatomical point is the absence of deep inguinal glands in the rabbit. According to Jossifow [1931] one or two superficial inguinal glands are present, but we have never seen these injected after introduction of colloidal dyes into the knee-joint. Deep inguinal glands are present in the dog, horse [Baum, 1920] and man. Some of the lymphatics draining the knee-joint in man enter the deep inguinal glands [Kling, 1938].

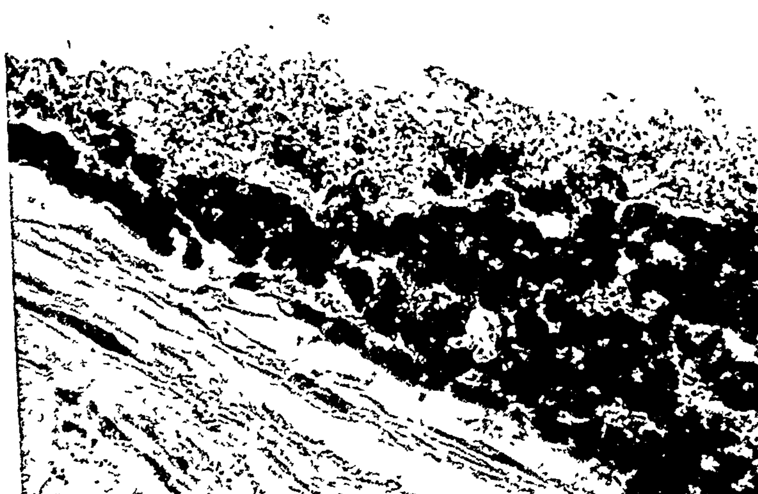
Absorption of True Solutions from the Joint Cavity.

Our experiments on the absorption of true solutions have been performed with uroselectan B (Schering), sodium iodide and methylene blue solutions. In the first two cases the absorption from the joint cavity has been followed radiographically. One cubic centimetre of uroselectan B injected into the knee-joint of a normal adult rabbit is absorbed in about 2 hours. Movement seems to have little effect on the rate of absorption in the normal animal and the time taken is not materially altered by immobilisation.

There appears to be no active absorption by the cells of the synovial

In the case of injections of 50 p.c. solutions of Higgin's India ink (size of particles 10–100 m. μ) 1 c.c. of the solution is removed com-

A



B

Fig. 2.—Low power (A) and high power (B) views of the synovial membrane of the knee joint of a rabbit 21 days after the injection of 1 c c. of colloidal mercuric sulphide into the joint, showing accumulation of injected particles immediately under the synovial membrane.

pletely from the joint cavity in about 10 days. The particles diffuse only into the subsynovial tissues, and a large quantity of the injected

system and have put particular stress on the rôle of movement in this removal. The following points also require consideration:—

1. The mode of removal of the particles out of the joint cavity into the surrounding tissue spaces.

2. The relative size of the particles that can be removed from the joint cavity or synovial membrane by normal lymphatic flow, as opposed to ingestion and carriage by phagocytes.

3. The question as to whether any colloidal particles from the joint cavity enter the blood-stream, and, if so, the relative size of particles so carried.

With these points in mind we have carried out numerous injection experiments into joint cavities, using Nile blue, trypan blue, Congo red, colloidal carbon, colloidal mercuric sulphide solutions and a finely divided bismuth suspension. There seems at present to be no means of determining the degree of aggregation of particles which may occur after the introduction of these solutions into the joint cavity and no means of preventing this aggregation without interfering with the results by producing further factors for consideration.

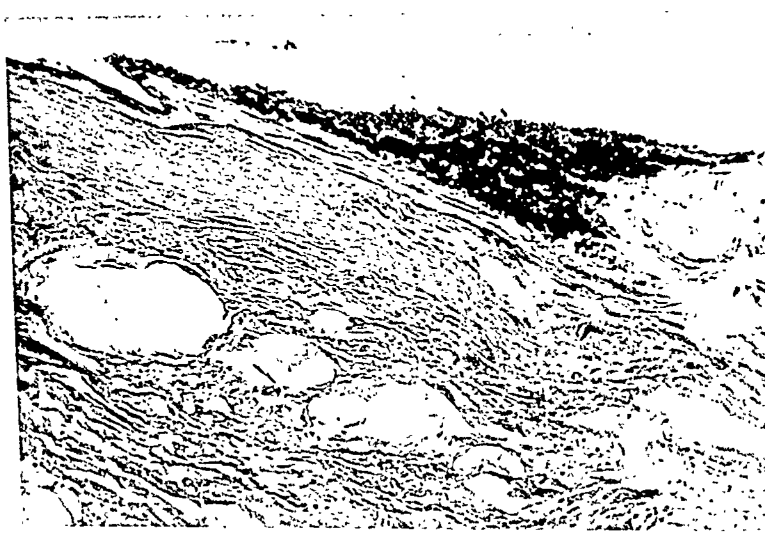
An injection of 1 c.c. of a 5 p.c. Congo red solution (molecular weight, 696) is removed from the knee-joint of the rabbit within 2 hours. Unmistakable diffusion into the surrounding fascial spaces occurs. Removal certainly occurs by the lymphatics as shown by the presence of the dye in the regional and abdominal lymph channels and in the popliteal and iliac glands at the end of the experiment. That marked absorption into the blood-vessels also takes place is shown by the following experiment.

An adult rabbit was kept anæsthetised during the whole of the experiment. The femoral artery, vein and lymphatics were ligatured in both femoral triangles and 1 c.c. of a 5 p.c. solution of Congo red was introduced into each knee-joint. The animal was killed at the end of $3\frac{1}{2}$ hours, when the blood was shown to be uniformly stained with Congo red although there was no evidence that this absorption had occurred by way of the abdominal and thoracic lymph channels. The lymph channels along the femoral artery were filled with dye only as far as the ligature.

With Nile blue solutions (molecular weight, 166) diffusion into the tissues around the joint is much more rapid and marked. Following the injection of 1 c.c. of a 1 p.c. solution into the knee-joint, the dye can be detected in the blood in 1 hour while it permeates along the lymphatics only as far as the level of the kidneys.

Results using a 1 p.c. solution of trypan blue (molecular weight, 564) follow closely those with Nile blue, the rate and degree of diffusion into the surrounding tissues being intermediate between those of Nile blue and Congo red.

In the case of injections of 50 p.c. solutions of Higgin's India ink (size of particles 10–100 m.μ) 1 c.c. of the solution is removed com-



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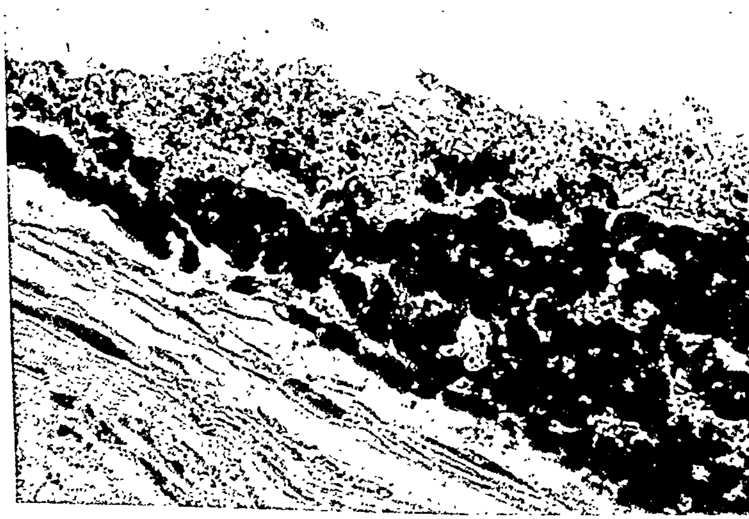

B

FIG. 2.—Low power (A) and high power (B) views of the synovial membrane of the knee-joint of a rabbit 21 days after the injection of 1 c.c. of colloidal mercuric sulphide into the joint, showing accumulation of injected particles immediately under the synovial membrane.

pletely from the joint cavity in about 10 days. The particles diffuse only into the subsynovial tissues, and a large quantity of the injected

material is seen along the sheath of the extensor digitorum longus tendon and muscle. A certain quantity is transported to the popliteal and iliac lymph glands. No evidence of absorption into the blood-stream can be seen.

With colloidal mercuric sulphide (size of particles, 100–200 m. μ) and the bismuth suspension (particles estimated average diameter 0·5 μ or more), no removal by lymphatics or blood-stream is observed, even after 17 weeks, although this does not prevent their removal out of the joint cavity. One cubic centimetre of the colloidal mercuric sulphide is removed from the knee-joint into the subsynovial tissue and the sheath of the extensor digitorum longus of the rabbit in 10 days (fig. 2). The particles remain in this situation longer than 17 weeks. The fate of the bismuth is similar (fig. 3). Mention may be made here of the fact that the colloidal mercuric sulphide does not induce any

A B

Fig. 3.—Radiographs of the right knee-joint of a rabbit immediately (A) and 116 days (B) after the injection of 0·75 c c. of a colloidal bismuth solution into the joint. The colloid is still recognisable in the tissues around the knee-joint, although the major portion of the bismuth has extended along the tendon of the extensor digitorum longus as far as the ankle.

visible "arthritic" changes in the joint even after 17 weeks. Marked "arthritic" changes, such as calcification of the synovial membrane and lipping of the articular margin, occur by 20 weeks after the injection of the bismuth suspension. In this respect one must bear in mind the necrobiotic action of bismuth.

Effect of Immobilisation.

The diffusion of the Nile blue, trypan blue and Congo red solutions is observed in the absence of movement of the joint and can be seen even in the recently killed animal, though not so markedly as in the living. This removal by diffusion occurs independently of movement and cellular action.

In the case of the lymphatic drainage of colloidal carbon particles, the effects of immobilisation of the joint are much more marked. No India ink particles are found in the lymph glands of the immobilised side even 2 days after the injection.

Discussion.

Bauer, Short and Bennett [1933] state that ". . . the size of the molecule which can be readily removed from a normal joint lies between that of horse serum albumin and horse serum globulin," *i.e.* 2–4 m. μ. Our experiments show clearly that particles as large as 10 m. μ at least escape from the joint into the subsynovial tissues. Furthermore, particles considerably larger than those of serum globulin pass from this situation into the regional lymphatic glands as evidenced by the passage of India ink particles. It would appear probable that globulin particles, whether as single molecules or as aggregates, share the same fate. There seems little need to postulate, as is done by Kling [1938], that the globulin particles may undergo disintegration by the proteolytic ferments which are present in joint fluids.

In the case of the rabbit the limiting size of particles which are eventually removed from the joint cavity into the lymphatic glands is that of mercuric sulphide (diameter 100–200 m. μ). This is in sharp contrast with the peritoneal cavity of which Bolton [1921] writes: "Particles may easily pass between the endothelial cells with the lymph, the limiting size of such particles being approximately that of the red blood cell of the animal used," and, "The blood corpuscles occur in small masses here and there in the peripheral lymph channel and in places in lymph sinuses."

Summary.

1. Removal of true solutions, colloidal solutions and fine suspensions from the joint cavity into the subsynovial tissues occurs comparatively rapidly. The rate is proportional to the size of the particle under consideration.

2. The mechanism of this removal is obscure. The experiments in this paper suggest a predominantly physical process, although an active rôle for the synovial cells has not been excluded.

The mechanism of further removal of substances from the subsynovial tissues varies with the size of particle.

(*a*) Small particles, *e.g.* true solutions, enter both capillaries and lymphatics with ease. Most are removed in the blood-stream.

(*b*) Small colloidal particles, *e.g.* Congo red, enter both blood capillaries and lymphatics, but with greater difficulty.

(*c*) Above a certain critical size, probably in the neighbourhood of the globulin molecule, removal by both these routes ceases, with the exception of small quantities which enter lymphatic vessels but are retained in the regional lymphatic glands.

(*d*) Still larger particles, *e.g.* 100 m. μ and over, have no route of egress from the subsynovial tissues.

We wish to express our gratitude to Professor H. A. Harris for his stimulating interest. Our thanks are also due to Mr. N. W. Pirie and Mr. R. Markham for their help and advice, and to Mr. J. A. F. Fozzard and Miss R. Ernest for technical assistance.

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THE DIFFERENCES BETWEEN BLOOD-PRESSURE MEASUREMENTS OBTAINED SIMULTANEOUSLY ON THE TWO ARMS.[1]

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(*Received for publication 6th December 1939.*)

Introduction.

Although differences in the arterial blood-pressure at different points of the circulatory system are known to exist, it is generally believed that in normal persons at rest in the supine position, differences in blood-pressure as determined on the two arms are less than the experimental error of the measurement. Consistent major differences between blood-pressure measurements on the two arms have, in fact, long been recognised as indication of the possibility of a deformity in or around one of the larger arteries [Osler and McCrae, 1935; Hills, 1938; King, 1937].

Moreover, in studies of clinically normal persons small differences in pressure measurements have been reported by Hensen [1900], Bing [1906], Fischer [1924], Geisböck [1905], Hare [1925–26], Hirsch [1901], McWilliam and Kesson [1912–13], Jellinek [1900], Bodenstab [1925], Kilgore [1914], and more recently by Yoshino [1933], Kay and Gardner [1930], and Korns and Guinand [1933].

In a study of the reliability of measurement of blood-pressure determinations [Shock and Ogden, 1939], it has been found that in most normal persons a measurable difference exists between simultaneous measurements made on the two arms. The details of experimental procedure and examinations of some other aspects of the data have already been reported [Ogden and Shock, 1938; Shock and Ogden, 1939].

Incidence and Magnitude of Difference.

For each of 102 subjects the average values of blood-pressure for each arm were computed from 48 simultaneous pairs of measurements (16 by each of three observers working two at a time in rotation).

[1] Grateful acknowledgment is hereby made to the Board of Research of the University of California for financial assistance in the collection of data, and to the Works Progress Administration (O.P. 65–1–08–62 Unit A–8) for statistical and clerical assistance.

The frequency distribution of the differences between these average values is presented in fig. 1, which shows that the average difference

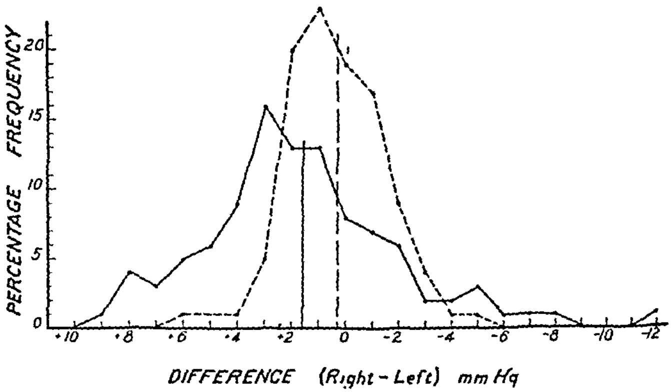

FIG. 1.—Frequency distribution of mean difference between blood-pressure determinations made simultaneously on right and left arms in 102 adult males. ——— systolic pressure differences; - - - - - - diastolic pressure differences. Vertical lines indicate mean of the distribution. (Systolic: $1\cdot60 \pm \cdot36$ mm. Hg; diastolic: $\cdot36 \pm \cdot18$ mm. Hg.)

(R–L) is $1\cdot60 \pm \cdot36$ mm. for systolic pressure and $\cdot36 \pm \cdot18$ for diastolic pressure.[1] The average value for right-arm measurements is higher in spite of one individual whose mean left-arm systolic pressure was

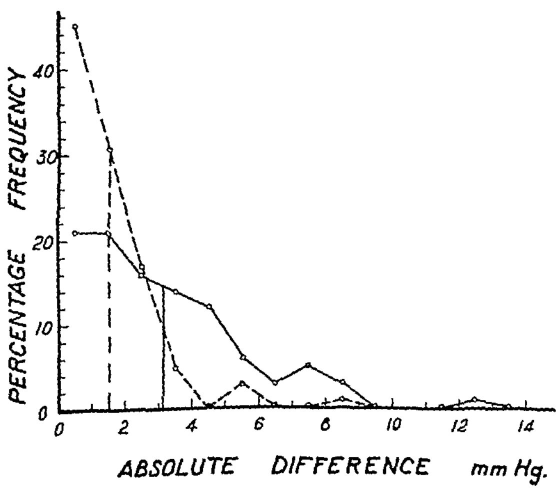

FIG. 2.—Frequency distribution of mean absolute difference (without respect to sign) between blood-pressure determinations made simultaneously on right and left arms in 102 adult males. ——— systolic pressure differences; - - - - - diastolic pressure differences. Vertical lines indicate mean of the distributions. (Systolic: $3\cdot1 \pm \cdot23$ mm. Hg; diastolic: $1\cdot5 \pm \cdot13$ mm. Hg.)

12·5 mm. higher than his right. In fig. 2, which presents mean differences without respect to sign, it may be seen that the absolute mean

[1] Throughout this paper standard error is used rather than P.E.

difference in systolic pressure is 3·1 ± ·23 mm. Hg and that 44 per cent. of the subjects show differences greater than the mean value. Even without the special facilities used in this study a difference greater

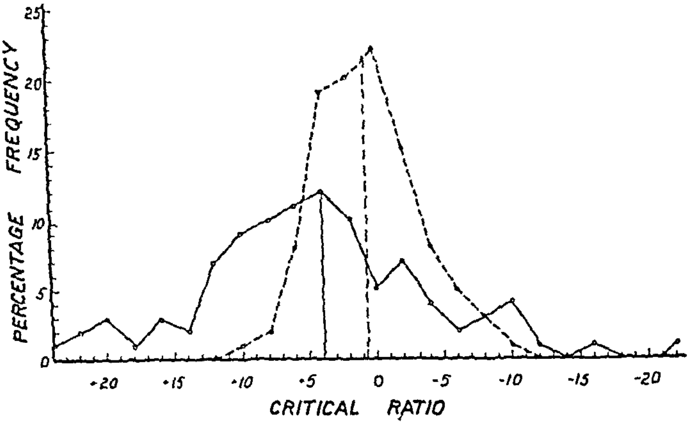

FIG. 3.—Frequency distribution of critical ratios of differences between blood-pressure determinations made simultaneously on right and left arms in 102 adult males. ——— critical ratios for systolic pressure differences; - - - - - - critical ratios for diastolic pressure differences. Vertical lines indicate the mean of the distributions. (Systolic: 4·0 ± ·9; diastolic: ·9 ± ·34.)

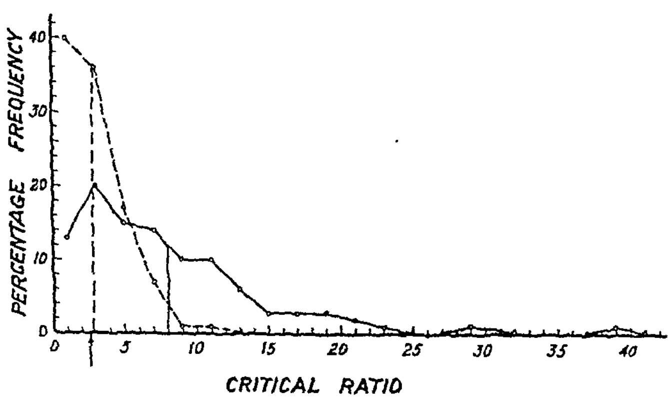

FIG. 4.—Frequency distribution of critical ratios of absolute difference between blood-pressure determinations made simultaneously on right and left arms in 102 adult males. ——— critical ratios for systolic pressure differences; - - - - - - critical ratios for diastolic pressure differences. Vertical lines indicate the mean of the distributions. (Systolic: 7·9 ± ·6; diastolic: 2·9 ± ·2.) Differences with critical ratios greater than 2·5 (see arrow) will occur by chance only 6 times in 1000.

than 3 mm. should be measurable. The mean difference without respect to sign for the diastolic pressure is 1·5 ± ·13 mm., which indicates a significant difference in the average.

In order to test the significance of these differences in individual subjects a distribution of the differences between simultaneous right- and left-arm measurements (R – L) was drawn up for each of the

102 subjects. From these distributions of 48 differences the standard deviation of the mean difference and the standard deviation of the actual distribution were computed for each subject. The ratio between this mean difference and its standard error could be readily computed, giving a critical ratio for each subject.[1] The frequency distributions of these 102 ratios are presented in figs. 3 and 4, which show that in 83 per cent. of the subjects the critical ratio was greater than 2·5 for systolic pressure and greater than 2·5 in 52 per cent. of the subjects for diastolic pressure. Critical ratios as high as 38 were obtained in some subjects for systolic pressures if the direction of the difference is ignored. The average C.R. was $7{\cdot}9 \pm {\cdot}6$ for differences in systolic pressures but only $2{\cdot}9 \pm {\cdot}2$ for differences in diastolic pressures.

Stability of the Difference.

That the difference does not consistently increase or decrease during an experiment is shown in fig. 5. Each point on this graph is the mean

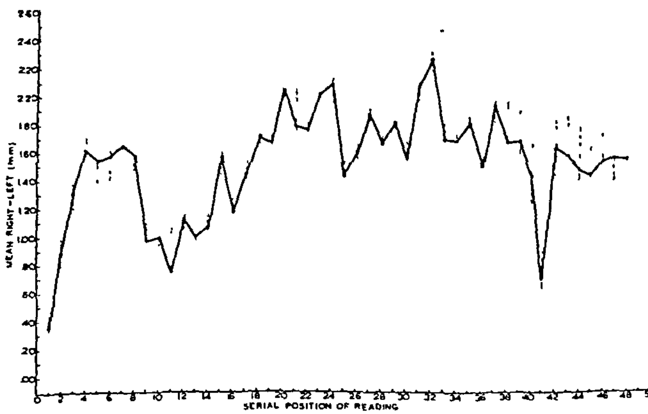

Fig. 5.—Mean difference between systolic blood-pressure measurements made simultaneously on two arms. The dotted lines indicate $\pm 1\sigma$ of the distribution of differences for 102 adult males. This figure shows the absence of any systematic relationship between the serial positions of pairs of readings and their mean differences. See text, p. 159.

value of one difference observation on each subject made at corresponding times during the experiment. Thus the first point is the average difference observed on the first pair of simultaneous observations on all subjects; the second point is the average difference on all subjects as observed 30 seconds later and so forth. Although the

[1] The results were checked by computing each critical ratio by the following formula as well:—

$$C.R. = \frac{Mn_R - Mn_L}{\sqrt{\sigma^2_{Mn_L} + \sigma^2_{Mn_R} - 2r_{RL}\sigma_{Mn_R}\sigma_{Mn_L}}}.$$

observers were changed after each 8 readings, the changes in mean difference values at these times are statistically insignificant; moreover, observations made by the three observers of this experiment have been shown to be without systematic difference [Shock and Ogden, 1939]. The average blood-pressure values were steadily falling during the first half of the experiment [Ogden and Shock, 1938], but the figure shows that this fall did not influence systematically the difference between the two arms. That the difference is independent of the time within the experiment and therefore of the temporal decrement of blood-pressure already discussed [Ogden and Shock, 1938] is further shown by the fact that the mean difference during the first half of the experiment was $1{\cdot}49 \pm {\cdot}36$, and for the second $1{\cdot}73 \pm {\cdot}34$.

Although the mean difference for the group was stable during the experiment, it appeared that the mean difference for all observations made on one individual during half an hour was not stable from month to month. This instability appeared when 40 of the subjects were retested after an interval of about a month. The correlations of the results of these two tests with respect to the differences of the mean measurements on the two arms of each subject and to the critical ratios of the mean differences are presented in Table I.

TABLE I.—CORRELATION OF RIGHT-LEFT ARM DIFFERENCES ON FIRST AND SECOND TESTS.

N = 40.

		Systolic.		Diastolic.	
		Mn.	*r.**	Mn.	*r.**
Mn. diff. R – L	Test I.	1·87	0·309	1·10	0·146
	Test II.	3·02	..	0·95	..
Critical ratio .	Test I.	4·15	0·315	3·12	0·148
	Test II.	2·47	..	0·68	..

* r = Pearsonian product-moment correlation.

The close similarity between the correlations obtained by these two methods (systolic $r = 0{\cdot}315$ and $r = 0{\cdot}309$) is interesting, and the low values for the correlations indicate that although differences were observed with approximately equal frequency in the two tests, the largest and most measurable differences were not shown by the same individuals in Test I. as in Test II.

Accordingly a table was prepared (Table II.) separating those individuals who never showed a significant difference from those

whose arms differed in Test I. only, in Test II. only, and in both tests.

TABLE II.—PER CENT. OF SUBJECTS SHOWING SIGNIFICANT RIGHT-LEFT ARM DIFFERENCES WHEN RETESTED.

N = 40. (Critical ratio of difference greater than 2·5.)

	Systolic.		Diastolic.	
	N.	Per cent.	N.	Per cent.
Test I. .	28	70*	11	27
Test II. .	29	72	7	17·5
Test I. or II. or both	36	95	15	37·5
Both Test I. and Test II.	16	40	2	5
Neither Test I. nor Test II.	2	5	5	12·5

* When computed for the 102 subjects taking the test for the first time, the proportion showing a significant difference was 83 per cent.

This table shows that, whereas there are only 40 per cent. of the subjects in whom the difference in systolic pressure appears in both tests and therefore might be due to a permanent anatomical characteristic, in 55 per cent. of them the phenomenon is liable, appearing only once in two tests; in only 5 per cent. (two cases) was there no significant difference in either of the two tests. It is apparent also that in any subject a significant difference is more commonly found in systolic pressure measurements than in diastolic.

Within the limitation imposed by a small group and only two experiments, we may conclude that it is very unusual to find someone whose systolic blood-pressure readings on the two sides never differ from each other.

DISCUSSION.

The common liability of the arm-to-arm differences revealed by these tests suggests that their major cause be sought in functional rather than anatomical peculiarities.

It is possible that the failure of the differences in blood-pressures of the two arms to distribute themselves about zero (mean systolic difference $R - L = 1{\cdot}60 \pm {\cdot}36$ mm. Hg) may be an indication of the expected hydrostatic pressure drop along the aorta between the origins of the blood supply to the two arms. The larger differences, however, which are characteristically neither stable nor favouring one arm, are more likely to be due to changes in the physical properties of the tissues

in the arm being measured. Thus an increase in rigidity or decrease in viscosity in the arm might very well allow a pulsation to become audible, the energy of whose sound might otherwise be dissipated. Such changes might be of vasomotor, muscular, or œdematous origin. The more obvious physiological methods for altering conditions in the two arms differentially have been repeatedly used [Hoover, 1910; Margaria, 1926, 1927; Mennyey, 1928; Yoshino, 1933] without a consistently predictable change in blood-pressure readings. For instance, Yoshino [1933] reports a lowering of blood-pressure in the exercised arm following unilateral arm exercise, but Margaria [1926, 1927] reports a marked rise in the exercised arm. Similar contradictions in results appear when temperature changes are used to induce differential vasomotor alterations in the two arms. Mennyey [1928] reported a rise in blood-pressure in the arm subjected to temperature rise; Hoover [1910] reported a rise in pressure in the arm subjected to cold. Such inconsistencies leave the field open to the speculation that such changes may so commonly be produced psychogenically as to mask experimental attempts to show them, except by the statistical analysis of a large number of physiological experiments in which a serious attempt had been made to randomise or exclude the possible psychological causes of the change. Within our knowledge such a series has not yet been published.

Summary.

Seventy per cent. of a group of normal young adult males showed a significant difference between blood-pressure measurements made simultaneously on the two arms. The distribution of the magnitude of these differences is presented.

The same proportion of significant differences was found in 40 of the subjects when retested. The correlation between the magnitude of the differences for each individual in the two tests was low, indicating that the cause of the difference in blood-pressure can rarely be a fixed structural characteristic of the individual.

Since these differences are not easily reproducible by variation of standard physiological conditions, it is suggested that they may be of psychogenic origin.

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whose arms differed in Test I. only, in Test II. only, and in both tests.

Table II.—Per Cent. of Subjects showing Significant Right-Left Arm Differences when Retested.

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	Systolic.		Diastolic.	
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Test I. or II. or both . .	36	95	15	37·5
Both Test I. and Test II. .	16	40	2	5
Neither Test I. nor Test II. .	2	5	5	12·5

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THE PROBLEM OF THE MOULT IN THE CASTRATED BROWN LEGHORN FOWL.

By Alan W. Greenwood and Marca Burns. From the Institute of Animal Genetics, University of Edinburgh.

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Introduction.

The annual shedding of the plumage followed by the growth of a new feather covering in birds is a problem of considerable interest, not only to the physiologist, but one which, because of the coincident depression in sexual activity in the domestic hen at this period, bears directly on economic aspects of poultry breeding.

From a series of observations on the moult in Brown Leghorn males, females and castrated males it was suggested by one of us [Greenwood, 1936] that the sex glands, although directly implicated, cannot be considered as causal factors in determining the occurrence of the phenomenon since in caponised males a definite and characteristic moult takes place in April, although the casting and renewing of the feathers continues until the autumn.

There is considerable disagreement amongst various workers on this question of the moult in the capon, and these views have been summarised by Benoit [1929]. "The capon does not exhibit the periodic annual moult, but, so to speak, a continual moult. The feathers continue to grow during the whole year, and at any one time one can see, in the different feather regions, new feathers forming. On this point I am in accord with Finlay [1925], but in disagreement with Pézard [1918] and Zawadowsky [1922]. Pézard finds no difference between the capon and the normal cock in regard to the moult. For Zawadowsky capons moult twice a year: in autumn (normal moult), and in January–February. I, for my part, have never noticed any true or characteristic moult in the numerous castrates that I have had occasion to follow for several years: I have always observed that young feathers did not cease to grow and form throughout the whole year." [1]

From this summary of views it will be seen that the accurate determination of the course of feather shedding in the capon still awaits

[1] Translation from the original French.

Hensen, H. (1900). *Deutsch. Arch. klin. Med.* **67**, 436.
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In addition, for comparison with the normal behaviour of intact birds, both males and females, a series of observations were recorded over a 20-week period from the 29/1/39 to 17/6/39 on 2 hens and 2 cocks.

RESULTS.

The Brown Leghorn male is a parti-coloured bird in which regional differentiation of feathers, both in regard to structure as well as colour, is well marked. The various feather types and their distribution on the body of the bird have been adequately described and illustrated by Chu [1938], and his classification has been adopted in this study. Although the regionalisation is clearly differentiated on the bird, a certain difficulty arose in classifying the cast feathers due to the close similarity between certain types from different regions and for this reason feathers are grouped according to colour and structure as follows:

- Group 1. Wing Bow. Cape. Shoulder. Saddle. Tail Coverts.
- Group 2. Sickles. Tail.
- Group 3. Wing Primaries. Wing Secondaries.
- Group 4. Fluff.
- Group 5. Under Tail. Thigh. Breast.
- Group 6. Hallux. Primary Coverts. Wing Coverts.
- Group 7. Throat. Neck Hackle.
- Group 8. Under Wing. Leg. Wing Front. Wing Contour.

The number of feathers shed by the experimental capons in a full year corresponded roughly with the number of feathers obtained from the bodies of the 2 control birds (Table I.). From this it follows that if annual feather replacement affects all follicles then the majority of feathers are shed only once during the year.

solution, and an attempt has been made therefore to obtain a quantitative measure of the mode and rate of moulting in these birds over a period of a complete year.

Material and Method.

For this investigation 3 adult Brown Leghorn males, castrated when less than 7 weeks old, were used. Previous to the experiment they had been kept intensively for some months in a battery unit of wire cages, and during the whole of this period they remained in excellent health. The three cages were adjacent and conditions of light and temperature, as well as feeding and general management, were essentially similar for all birds. Food and water dishes were affixed to the outside of the cages so that it was never necessary to handle the birds during the course of the experiment. Two of the capons were hatched in the spring of 1935 and the remaining one on 1st June 1936.

The cast feathers were collected from the wire-mesh floors of the cages and the trays beneath them at frequent intervals. Observations were commenced on 12th October 1937 and continued until 10th October 1938. In order to minimise the chances of accidental loss the feathers were collected every morning when possible. During the summer of 1938 collection was unavoidably less regular, and it is possible that some loss of feathers occurred during this period, although it is doubtful if such losses were of sufficient magnitude to affect the general results.

From experience it has been found that fowls strictly confined tend to exhibit depraved appetites in spite of the provision of adequate nutritional requirements. This depravity takes the form of feather eating and may occur either as a result of direct cannabalism when feathers are plucked out and eaten, or by the ingestion of feathers from the floor of the pen or cage. In the latter event the habit is confined to growing feathers prematurely cast and which have a vascular base; eating of mature feathers has not been observed to occur to any appreciable extent, and therefore this cannot be considered to be a potential source of loss in the investigation.

It is suspected that the very small feathers found in the head and throat region may have been underestimated owing to the probability that some of these were intimately mixed with the considerable amounts of fæcal matter in the trays.

In order to obtain a quantitative determination of the rate of feather shedding it was necessary to estimate the total number of feathers in the plumage. This was done by killing 2 control capons in the month of November (a time which will be seen subsequently to exhibit a low proportion of cast feathers), and then removing, classifying and counting the feathers.

a significant reduction in number until the end of the series of observations. These figures indicate that a well-marked spring moult can be observed in capons if an intensification of the rate of feather loss be adopted as a criterion of moulting. In the particular sense that, since the birds are losing feathers continuously throughout the year and therefore exhibit a persistent moult, the findings of Benoit are valid, but, superimposed on this is a very definite seasonal change in rhythm not implied in his statement, a phenomenon of considerable importance since it leads to the assumption previously made by Greenwood of the probability of capons exhibiting a characteristic moulting time in the absence of sex glands.

TABLE II.

AVERAGE MONTHLY PERCENTAGE OF TOTAL FEATHERS CAST.

4-week periods.	Per cent. of total.	Per cent. in discrete feather groupings.							
		1.	2.	3.	4.	5.	6.	7.	8.
12/10–7/11	2·7	0·7	8·5	3·0	2·1	0·3	1·3	5·1	0·6
8/11–5/12	3·1	1·6	6·7	1·7	1·7	..	3·5	6·5	0·7
6/12–2/1	2·3	0·7	11·6	1·7	0·9	0·2	1·3	5·1	1·1
3/1–31/1	3·1	0·6	11·6	6·5	1·6	0·2	2·6	6·5	1·2
1/2–28/2	3·2	1·2	3·3	3·0	2·3	1·0	1·3	5·4	2·7
1/3–28/3	8·0	4·8	6·7	6·5	9·0	1·3	6·5	10·4	4·0
29/3–25/4	10·1	11·4	1·7	6·5	11·2	1·9	17·4	9·9	8·5
26/4–23/5	10·3	34·1	5·0	16·3	7·4	1·7	14·8	3·3	17·0
24/5–20/6	20·4	22·7	11·6	22·8	20·8	27·9	27·0	11·0	40·2
21/6–18/7	18·3	9·1	11·6	14·7	24·7	30·8	13·5	14·5	15·1
19/7–15/8	8·8	5·8	6·7	9·3	10·5	19·2	3·9	8·3	4·3
16/8–12/9	4·8	4·1	..	4·0	4·1	8·5	1·7	6·5	2·2
13/9–10/10	4·9	3·2	15·0	4·0	3·7	7·0	5·2	7·5	2·4
Average No. of feathers per bird:	7138	875	20	57	2634	298	77	2352	825

It is to be expected that, as in the hen, there will be a range of variation in the time at which the individual capons exhibit their maximum feather loss. (The onset of the moult in the female is readily determined since, in the majority of birds at least, there is a coincident cessation of egg production. In the male no such indicator of the moult exists and its onset must be determined by direct observation.) Two of the capons behaved similarly with their maximum amount of feather shedding in the 9th period, while the 3rd bird showed marked intensity in discarding feathers in the 7th period (29/3–25/4). There is thus some

Table I.

Feather Groups.	Experimental capons. Number of feathers shed.				Control capons. Number of feathers on body.		
	No. 1.	No. 2.	No. 3.	Average.	No. 1.	No. 2.	Average.
1	936	942	748	875	857	850	853
2	13	35	11	20	18	14	16
3	64	61	46	57	60	58	59
4	2260	2729	2914	2634	2069	2403	2236
5	273	369	253	298	501	548	525
6	63.	90	77	77	84	78	81
7	2515	2355	2186	2352	1501	1765	1633
8	649	944	883	825	1266	1534	1400
Totals	6773	7525	7118	7138	6356	7250	6803

Closer examination of the figures for the different feather groups shows that the number in 5 and 8 in each of the experimental birds is lower than would have been expected, while the number in Group 4 is considerably larger than that derived from the control birds. It is not considered that this represents a difference in the extent of the moult in the first two groups, but rather that the effect may have been produced by the inclusion of many feathers from these in with Group 4 since many of them from these regions are structurally very similar to fluff feathers.

The only other well-marked deviation in number between the two sets of birds is shown by Group 7. The significance of this will be dealt with later, but it can be noted at this point that this region possesses the smallest feathers to be found on the body of the bird (the head area), and it is therefore reasonably certain that many of the cast feathers have been lost and the apparent difference in feather number should actually be much greater than that recorded.

Having determined that the total number of feathers cast by capons during the course of a year approximates to the estimated number comprising the plumage, the rate of moulting may now be examined from the relevant data given in Table II.

From the data it is clear, if monthly totals averaged from the 3 birds are considered, that from the beginning of the observations in October up to the end of February the rate of casting feathers was the same, viz. approximately 3 per cent. of the total annual number were shed in each of the first 5 time groupings. From the beginning of March, however, the percentage of shed feathers increases rapidly until a maximum is reached in the 9th period (24/5–20/6); this is followed by

deeply on the underlying causes of this variable behaviour in the different body regions, but it is suggestive perhaps that the feathers of the breast, indicating by their structure and colour a hypersensitivity to thyroid stimulation [Greenwood and Blyth, 1929], should remain relatively unaffected in the inter-moult period while those of the neck region, typical hypothyroidic feathers, should respond continuously to the forces controlling moulting of the plumage.

In order to obtain a comparison of normal adult birds with capons, 2 males and 2 females were similarly confined in battery cages and the cast feathers collected over a period of 20 weeks, commencing on the 29th January and ending on the 17th June. A summary of the findings is given in Table III.

TABLE III.—COMPARISON OF THE AVERAGE NUMBER OF FEATHERS SHED BY NORMAL MALES (2), NORMAL FEMALES (2), AND CASTRATED MALES (3), IN A 20-WEEK PERIOD.

Feather group.	Average number of feathers lost.		
	Males.	Females.	Capons.
1	2·5	11·5	649·3
2	1·0	..	5·7
3	..	0·5	31·3
4	13·0	107·0	1334·0
5	2·5	18·5	100·7
6	..	1·5	51·3
7	1·5	45·5	940·0
8	1·5	11·0	587·0
Total average No. per bird	22·0	195·5	3709·3
Estimated percentage of plumage (6803 feathers)	0·32	2·87	54·52

The normal male and female obviously behave very differently from castrated males. In the period the birds were under observation the males have lost comparatively few feathers, while the females, though somewhat higher numbers were recorded, yet show a loss for the 20 weeks comparable with a single 4-week period only during the season of minimum feather casting in capons. It is striking that while feather losses from the intact birds were so small, the capons lost more than fifty per cent. of their feathers in the same period of time.

indication that all capons would not behave similarly and that the intensity of the moulting processes is not directly referable to seasonal effects but suggests the operation of an inherent rhythm.

From Table II. it may also be seen that the intensity of the moult varies in time in the different regions of the body, and this effect has been graphically recorded in fig. 1.

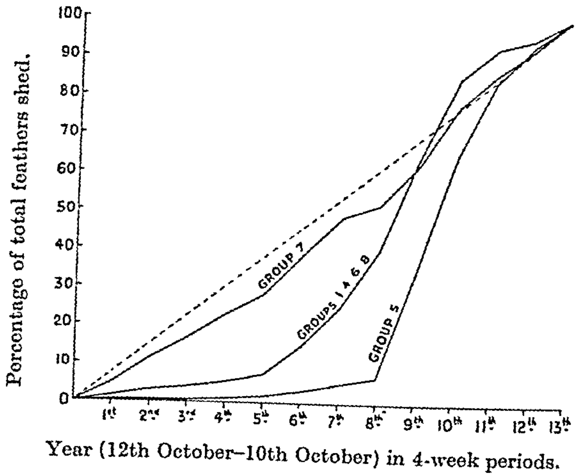

Fig. 1.—To show regional differences in the mode of moulting feathers in capons. Broken line indicates the theoretical conception of a continual and constant rate of moulting.

Because of the relatively small numbers of feathers in the flights only the regions composed of contour feathers have been utilised. The graph shows that there are 3 types of regional moult. In Group 7 (head and neck hackle) there is a continuous shedding of feathers at a comparatively high rate throughout every period of the year, with one exception, the 8th. Of all regions this behaves most like that characterising a hypothetical continual moult (broken line in graph), and approaches Benoit's conception of the moult in castrates. The absence of feather shedding to any appreciable extent in the 8th period (shown by all 3 birds) is the only evidence found in support of Zawadowsky's hypothesis that such birds moult twice a year. That the neck region is particularly susceptible to moulting is the common experience of all poultry-keepers, and such a phenomenon is not to be confused with the true moult affecting all regions of the plumage.

The region of the body reproducing most clearly the moult as it apparently affects intact birds is the breast (Group 5). Few feathers are shed until the 9th period when a vigorous casting of feathers is observed. The remaining regions (Groups 1, 4, 6, 8) show an intermediate type of behaviour between those previously referred to.

From the limited data available it would be unwise to speculate too

[Hill, Corkhill and Parkes, 1924], but while the primary factor here may be the removal of the pituitary gland it is significant that the new feathers subsequently growing in indicated by their colour and structure a marked inactivity of the thyroid glands.

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Summary and Conclusions.

From the observations that have been recorded here on the shedding of feathers from the plumage of fowls it can be concluded that capons do not behave in the same way as normal males and females. They exhibit a tendency, to an appreciable extent, to cast feathers in the winter period, a season of the year when normal birds retain their feathering.

With the onset of spring an intensification of the moulting process sets in and feather loss reaches its maximum in the May–June period, and this may be taken as the appearance of a true moult in such birds. Females, on the other hand, characteristically enter the moult much later in the year—autumn and early winter—as determined by the cessation of egg production and coincident casting of feathers. In normal males, although no accurate determination of moulting phenomena has so far been made, casual observations over a number of years suggest that they tend to moult at a time intermediate between that shown by castrates and females respectively.

The data discussed lend support to the hypothesis previously put forward by Greenwood that in the fowl reproductive activity inhibits the onset of the moult, and only when this activity ceases, or continues at a lower level, can the casting and renewing of feathers occur.

The investigation failed to provide evidence in support of the conclusion of Benoit [1929] that the capon does not exhibit a true annual moult, although agreement is reached with him on the point that feathers continue to grow and are replaced throughout the whole year. The contention of Pézard [1918] that capons behave as normal males received no confirmation, and the view of Zawadowsky [1922] that castrated males moult twice a year was not borne out, neither by reference to the total number of feathers shed by these birds in the course of a full year nor by the rate of feather shedding. His explanation of the phenomena would only be valid if confusion had arisen with regard to the moult as it affected one particular region only, the neck, an area which, even in normal birds, may exhibit a double moult annually.

A further point of interest which was brought out in this study is the appearance of distinct and characteristic rhythms in feather casting in various regions of the plumage. It has been suggested tentatively that an explanation of this may be found by attributing to the expression of a causal relationship the known sensitivity of certain feather types to thyroid stimulation with the variable character of the moult as it affects certain regions. Among many workers Giacomini [1924] and Zawadowsky [1925] have shown that a generalised moult may be obtained in birds by feeding a heavy single dose of thyroid substance. Moulting of the plumage follows as a direct result of hypophysectomy in birds

THE PHARMACODYNAMICS OF THE DOMESTIC FOWL WITH RESPECT TO ERGONOVINE AND ERGOTAMINE.

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INTRODUCTION.

THE relatively few pharmacological studies on the domestic fowl (*Gallus domesticus*) stand in marked contrast with the numerous physiological and nutritional studies which have been reported. Of historical interest are the experiments of Paracelsus [1658] on the hypnotic effects of ether on chickens. In more recent times Sharpe [1912–13] studied the secretion of urine in hens and found that they responded to the common diuretics in a manner similar to the more conventional laboratory animals. He experienced some difficulty in measuring avian blood-pressure. Hogben and Schlapp [1924], Hogben [1925], and Gaddum [1928] investigated the depressor response obtained with pituitary extracts in the fowl. Later it was shown by Morash and Gibbs [1929] that this action was a property of the oxytoxic fraction. Coon [1939] has proposed the use of this depressor response as a method of assaying the oxytoxic activity of posterior lobe extracts. Gibbs [1926, 1928, 1929] in his report on the action of various drugs on circulation in the chicken includes the information that ergotamine will give a prolonged rise in blood-pressure in this animal and in some instances an epinephrine reversal. He measured blood-pressure from the "popliteal" artery and rarely experienced difficulties.

Several workers have been concerned with the action of drugs on various isolated organs of the domestic fowl. These include Morash and Gibbs [1929] on the intestine, McKenney, Essex, and Mann [1932] on the oviduct, and Coon [1939] on the heart.

A few of the present bioassay methods use chickens. Among those which might be mentioned are chicks for antirachitic substances and gonadotropic and thyrotropic hormones, roosters for official ergot preparations, and capons for the male sex hormones.

The infrequency with which the fowl has been used is surprising when one considers the many advantages which it possesses over other laboratory animals. Conspicuous among these are its availability the

at any time. Artificial respiration when needed was administered by a Palmer pump. The blood-pressure was recorded by means of a mercury manometer connected to the ischiadic artery, and injections were made directly into the crural vein. A description of the technique employed is given by Coon [1939].

Ergonovine elicited a pressor response in thirty-seven of the chickens. A significant rise was obtained with amounts as low as 0·004 mg. (fig. 1),

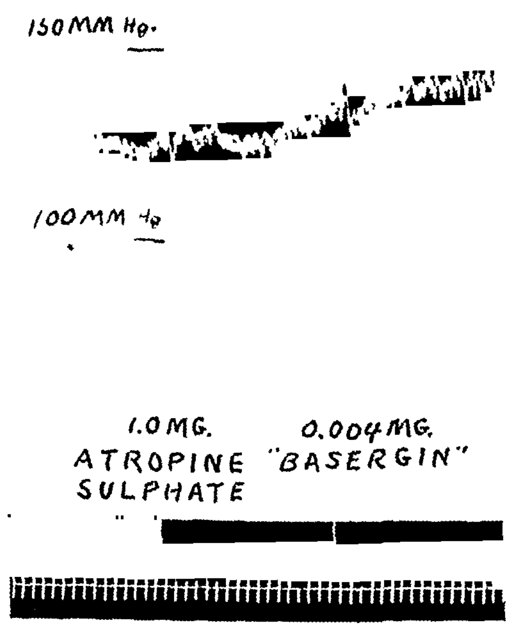

FIG. 1.—16.12.38. White Leghorn cock, wt. 2·18 kg. Phenobarbital-Na 200 mg./kg. I.M. Vagi cut. 38–V–R.
Blood-pressure response to a minimal dose of ergonovine.

and definite suggestions of a rise were obtained with only one-half the amount.[1] This quantity is considerably less than that reported necessary (0·1–1·0 mg.) to obtain blood-pressure changes in cats, dogs, or rabbits. When given to the fowl 0·1 mg. of ergonovine usually caused an elevation in pressure of some 30 mm. Equal doses of ergonovine and ergotamine produced approximately equal rises in blood-pressure, except in one or two cases in which the response to ergotamine was nearly twice that of ergonovine. This rise was quite prompt, occurring 10 or 15 seconds

[1] All quantities have been expressed in terms of total dose, not dose per kilogram.

year round at moderate cost, its docility, its modest housing requirements, and finally its meagre emotional appeal to persons opposing vivisection. The most serious criticism which can be directed against the fowl is its distance from man on the phylogenetic scale, so that information obtained on it may not be assumed to apply to human biology. However, any animal experimentation is open to similar objection to a greater or lesser degree.

The present study of ergonovine and ergotamine on the fowl (White Leghorn) was undertaken both to add to the information concerning these alkaloids and to re-emphasise the practicability of utilising this somewhat neglected animal. No reports comparing the effects of these drugs on chickens have been found other than those dealing with toxic manifestations [Brown and Dale, 1935] and comb darkening [Brown and Dale, 1935; Thompson, 1935].

Materials Employed.

The alkaloids employed were the maleate and tartrate of ergonovine, namely "Ergotrate" (Lilly) and "Basergin" (Sandoz), and the tartrate and methanesulphonate of ergotamine.[1] Since no differences in the biological response to the first two salts were found, the term "ergonovine" is used in this paper to refer to either the maleate or tartrate of the alkaloid. The term "ergotamine" is used instead of ergotamine tartrate for the sake of simplicity. Usually solutions were prepared from the solid material in quantities sufficient for two or three days' use. These were stored in a refrigerator. The solvent was 0·9 per cent. sodium chloride solution. In some instances sterile ampules of the alkaloids were employed. The ergotamine tartrate used in many of the experiments was a stock solution of "Gynergen." In no case could any variation in response be attributed to the type of solution in which the various alkaloids were administered. The pH of the ergonovine solutions was about 5·6, while that of the ergotamine tartrate solutions was about 3·4. Control injections included physiological saline, sodium tartrate, and tartaric acid.

Blood-pressure Studies.

Thirty-five of the forty birds used in this part of the work were roosters, since they appeared to have a more uniform blood-pressure than hens. Sex, however, did not appear to be a determining factor with regard to the hemadynamics of ergonovine and ergotamine. Sodium phenobarbital, 200 mg. per kilogram intramuscularly, was used in all but one experiment. In this isolated instance a local anæsthetic (procaine) was used. No supplementary anæsthetic agent was required

[1] For the generous supply of these materials acknowledgment is made to the Eli Lilly Research Laboratory and to the Sandoz Chemical Works.

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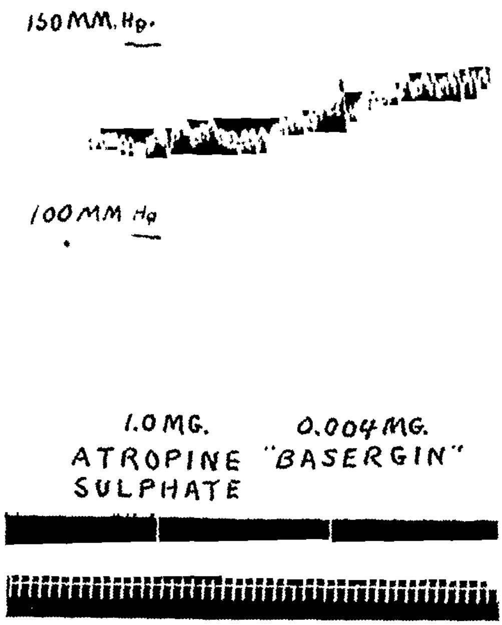

FIG. 1.—16.12.38. White Leghorn cock, wt. 2·18 kg. Phenobarbital–Na 200 mg./kg. I.M. Vagi cut. 38–V–R.

Blood-pressure response to a minimal dose of ergonovine.

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following injection. In preparations in a state of shock the interval between injection and response was slightly longer, due presumably to sluggish circulation. In general the pressor effect of ergonovine was much more evanescent than that of ergotamine (fig. 2). This observation parallels that of the comb-darkening action of the ergot alkaloids,

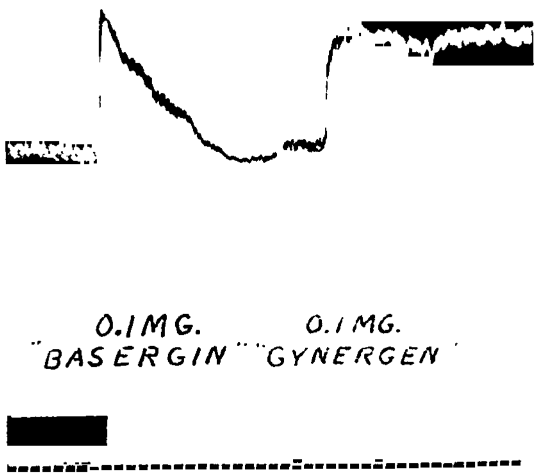

FIG. 2.—16.12.38. White Leghorn cock, wt. 2·0 kg. Phenobarbital-Na 200 mg./kg. I.M. 38–V–R.

A comparison of the pressor responses to ergonovine and ergotamine.

in which ergonovine has been reported to produce a prompter but more temporary darkening than ergotamine.

In three of the birds a fall in blood-pressure was observed with ergonovine although ergotamine elicited a rise (fig. 3). Another salt of ergonovine, the hydracrylate, and the free base itself in the form of "Ergometrine," also depressed the blood-pressure in these roosters. These three roosters did not appear abnormal and presented no peculiarities in their response to other drugs.

That the pressor effect obtained with ergonovine in the majority of cases was not an artifact of general anæsthesia was indicated by the results in the chicken when 0·1 per cent. procaine-hydrochloride infiltration anæsthesia was employed. Both alkaloids in the usual dosage range raised the pressure in a manner similar to that in fowls under phenobarbital anæsthesia. Vagotomy did not alter the pressor response in the chicken. Large doses of atropine (9 mg.) also left this response unchanged. The rise in pressure was slightly less in the decapitate

fowl, and a response was obtained even after pithing the cord. These experiments indicate that while most of the action is peripheral the central nervous system is responsible for at least part of it.

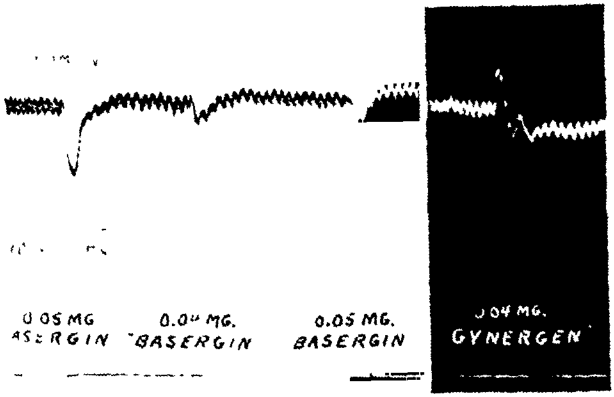

FIG. 3.—9.1.39. White Leghorn cock, wt. 1·75 kg. Phenobarbital-Na 200 mg./kg.
I.M. 44–V–R.
Fall in blood-pressure following ergonovine.

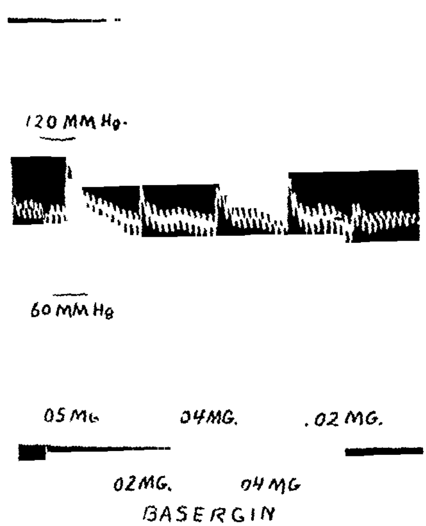

FIG. 4.—29.10.38. White Leghorn hen, wt. 1·9 kg. Phenobarbital-Na 200 mg./kg.
I.M. 5–V–R.
Pressor responses to varying doses of ergonovine.

With doses of ergonovine less than 0·1 mg. a fair correlation was found between the pressor response and the amount of the material injected (fig. 4). This fact in conjunction with the greater sensitivity of the chicken suggests its use as a means of checking the potency of solutions known to contain ergonovine.

Some variability in response to ergonovine was observed in this series other than the fall in blood-pressure noted in three cases. Although the pressure usually returned to its previous level quite promptly (3–6 minutes) following an injection of about 0·05 mg. of ergonovine, in some chickens the return was gradual, lasting from 8 to 12 minutes. In a few others the return was prompt, but was followed by a secondary rise which lasted about 5 or 6 minutes and was usually less than half the primary rise. In two instances injections of ergonovine following ergotamine elicited a weaker response than an equal dose given just before. On one occasion the injection of ergonovine following ergotamine produced a fall in blood-pressure, whereas this chicken gave pressor responses at other times during the experiment.

In the phenobarbitalised chicken a rise in blood-pressure followed doses of epinephrine-hydrochloride as small as 0·2 μg., and the responses were fairly proportional to the amount of the drug given. In no case was it possible to obtain an epinephrine reversal following ergonovine, but an inhibition of the epinephrine response following ergonovine in amounts from 10 to 20 mg. was frequently observed. In some instances the response was nil, and in others it was definitely less than the epinephrine response preceding the ergonovine. After a recovery period of about 20 minutes the response to epinephrine was usually of about the same magnitude as before ergonovine. These observations parallel those of Brown and Dale [1935], who found that following large amounts of ergometrine (ergonovine) the pressor response of the spinal cat to epinephrine was much reduced but not reversed.

In the fowl epinephrine following ergotamine produced less of a rise in pressure than when given before, but in no instance was a reversal obtained. It is possible that the mild atropine-like action of phenobarbital was in part responsible for the absence of reversal following ergotamine [Herwick, Linegar, and Koppanyi, 1939]. The familiar cocaine sensitisation to epinephrine was readily demonstrated in the chicken.

Besides the circulatory changes in these anæsthetised fowls, ergonovine and ergotamine in doses as small as 0·01 mg. produced momentary respiratory depression. This was characterised by shallower breathing and a decrease in rate. Occasionally the depression was followed by a brief period of tachypnœa. The respiratory depression appears to be a primary effect of these drugs and not a result of reflexes caused by elevated blood-pressure, for the inhibition of respiration occurred even when ergonovine produced a depressor response. Quantities of the drugs sufficient to inhibit the response to epinephrine usually caused respiratory failure. In such instances artificial respiration was employed. The respiratory effects of ergonovine on the fowl are substantially the same as those reported for etherised cats [Davis, Adair, Chen, and

Swanson, 1935], in which doses of 5 to 30 mg. greatly suppressed the rate and amplitude and caused cyanosis and circulatory depression which were relieved by artificial respiration.

Electrocardiographic Studies.

In order to demonstrate possible effects of ergonovine and ergotamine on the conducting mechanism of the heart, electrocardiograms were taken on phenobarbitalised chickens. Lead III was used in most cases. The electrodes were attached to the biceps region of each wing and to the tibial region of the left leg.

An injection of 0·05 mg. of ergonovine in one instance reduced the heart-rate from 188 to 162 beats per minute but did not otherwise alter the characteristics of the electrocardiogram. In another chicken the same amount changed the rate from 161 to 132 beats per minute. After decapitation the rate was only reduced from 172 to 164 beats per minute by 0·1 mg. of ergonovine. Following pithing of the cord this quantity increased the rate slightly. Ergotamine, 0·05 mg., decreased the heart-rate from 171 to 145 beats per minute. Following decapitation there was practically no change in rate with this dose. The electrocardiogram showed no changes other than that of rate. A large dose of ergonovine, 5 mg. per kg., slowed the heart and flattened the T-wave slightly. There were no other changes. Ergotamine in the same dose also slowed the heart, but in addition caused the T-wave to become diphasic. The P–R interval and QRS complex were not altered.

Pitocin, pitressin, epinephrine, neosynephrin, benzedrine, and ephedrine in amounts which produced moderate changes in blood-pressure had no effect on the electrocardiogram aside from minor changes in the heart-rate.

Experiments on Isolated Organs.

Heart.

Certain peculiarities in avian anatomy and physiology had to be considered in working with the isolated heart. In the first place the large sternum hinders one in getting at the heart. Additional delay is caused by two large branches which leave the aorta very close to the heart and which have to be ligated before perfusion can be started. The asphyxia resulting from these delays is often sufficient to produce irreversible damage. It was found that exposing the heart under either general anæsthesia with sodium-phenobarbital, or local anæsthesia with 5–10 c.c. of 0·1 per cent. procaine-hydrochloride, facilitated removal and produced a much more satisfactory preparation than could be obtained from chickens rendered unconscious by breaking the neck. The Ringer-Locke perfusion fluid was adapted to the physiology of the chicken by increasing its osmotic pressure (NaCl 0·95 per

cent.) and temperature (42° C.). The coronary flow in the isolated chicken heart was found to be two or three times that of the rabbit or cat and consequently exceeded the capacity of conventional drop recorders. An apparatus was designed which would handle this large volume and yet respond to fleeting changes in flow which are obscured by a bucket-type recorder. This device consisted of a stopcock which diverted a constant portion of the flow and permitted the remainder to fall as drops and actuate an electric recorder. These drops, together with the contractions of the heart, were recorded on a kymograph.

Hearts prepared in this manner and perfused at a pressure of 60 cm. beat vigorously from 1 to 4½ hours, permitting the study of the effects of drugs added to the perfusion fluid just before it entered the coronaries. They did not, however, beat as long or as uniformly as cat and rabbit hearts which were used for comparison.

The most striking effect of ergonovine and ergotamine was found to be on the coronary flow. In every one of the 15 chicken hearts studied the first dose of either drug caused a marked reduction in flow. In the case of ergonovine quantities as small as 0·05 mg. produced a decrease of 25 to 35 per cent. Ergotamine appeared to be some ten times more potent. With larger amounts of either drug still greater diminution in coronary flow was obtained, as high as 43 per cent. with 0·1 mg. of ergonovine and 68 per cent. with 0·01 mg. of ergotamine. With ergonovine this decrease in flow lasted 10 to 15 minutes before a return to normal occurred, while with ergotamine recovery was slower and only rarely did the flow ever return to the initial level. In one heart an initial dose of 0·1 mg. of ergotamine reduced the coronary flow to 33 per cent. of its original rate, and although over 2 hours were allowed to elapse without any additional drugs being given, the flow was still only half of what it had been at the start. It should be noted in this connection that the ergotamine solution used was acid, and that the alkalinity of the perfusion fluid was sufficient to neutralise this and tended to precipitate out the ergotamine. The very persistent action of ergotamine is probably not due to a mechanical obstruction of the capillaries with precipitated material, since a degree of experimental air embolism sufficient to cause equivalent reduction in flow was accompanied by marked disturbances in the rhythm and amplitude of the heart such as were not observed with ergotamine. The low solubility of the alkaloid at a physiological pH may well contribute to its prolonged action by delaying its diffusion out of tissues once it has penetrated them. The possibility of the decreased coronary flow being due to a passive constriction of the capillaries by increased ventricular tone is excluded by the characteristic response to ergonovine and ergotamine obtained in hearts which had ceased to beat. The response of the coronary flow to these two drugs seemed to be independent of the type of anæsthetic used.

Only rarely were subsequent doses of these two alkaloids as effective as the initial one in reducing coronary flow. In the case of ergotamine this may be ascribed chiefly to the fact that the rate of flow was permanently reduced, and tended with each dose to approach a limit beyond which further slowing was impossible. With ergonovine, however, even though recovery was fairly rapid and complete, repeated administration gave evidence of tolerance. Thus an initial 0·05 mg. of ergonovine reduced the coronary flow from 37·2 to 27·0 c.c. per minute. One half-hour later the flow had returned to 41·2 c.c. per minute, and a second dose of 0·05 mg. of ergonovine only decreased it to 36·1 c.c. per minute. This is a 50 per cent. reduction in effectiveness.

There was definite evidence of cross tolerance in that when ergotamine was the first drug given to a heart; later doses of ergonovine were less effective in reducing the coronary flow than equivalent doses given to a fresh heart. Evidence of tolerance to ergotamine produced by ergonovine was suggestive but not as consistent. It is evident from a consideration of these results that a satisfactory comparison of the potency of these two alkaloids in reducing coronary flow is possible only by using a series of hearts and comparing the effects of the first drug given to each.

About half the hearts responded to ergotamine or ergonovine at some time during the experiment with a transitory increase in flow. This increase lasted about 10 seconds and was always immediately followed by the larger and more characteristic decrease. The increase was on the order of 20 per cent. It occurred most consistently when relatively large amounts of the drugs were used, *e.g.* 0·5 mg. or more of ergonovine. There was suggestive but not conclusive evidence that hearts removed from chickens under local or general anæsthesia were refractory to this action of ergonovine and ergotamine. The acceleration in coronary flow is not a part of a generalised sympathetic stimulation of the heart, as it can occur without any change in the rate or amplitude of the ventricular contractions.

The other actions of ergonovine and ergotamine on the isolated heart were inconspicuous in comparison with the effects on the coronary flow. Even with doses as large as 2·2 mg. of ergonovine and 0·1 mg. of ergotamine only minor unpredictable changes in the amplitude of contractions were obtained. Effects on frequency of contractions were equally irregular, with the exception of the tendency of an initial dose of ergonovine to accelerate hearts removed from phenobarbitalised chickens. It seems probable that the capricious action of these alkaloids is due to a mixture of sympathetic and parasympathetic effects [Gayet and Minz, 1936; Navratil, 1937], and that a mild parasympathetic depression resulting from phenobarbital [Linegar, Dille, and Koppanyi, 1936] may have permitted the sympathetic action to predominate.

Seven additional drugs (calcium chloride, epinephrine, ephedrine,

neosynephrin, tyramine, pitocin, and pitressin) were administered to isolated chicken hearts, but since their action on mammalian hearts is well known, and their behaviour on that of the chicken showed no unusual features, no detailed results will be presented. They served only to demonstrate that the avian heart responds characteristically to ordinary drugs. Similarly repeated doses of ergonovine and ergotamine were administered to 4 cat and 4 rabbit hearts. Since Donatelli [1938 *a*, 1938 *b*, 1938 *c*] has reported on the complex action of ergotamine and ergonovine on the mammalian heart, mention need be made here only of the effects on the coronaries, a phase apparently not included in his researches. It was found that the results obtained on cat and rabbit hearts paralleled in the main those already described for the fowl. Thus it was possible to demonstrate the prolonged decrease in flow, the initial transitory increase with large doses, and the development of tolerance. Experiments were not sufficiently numerous, however, to convincingly demonstrate such a complex relation as cross tolerance. Neither can any statement be made concerning the influence of preliminary anæsthesia since all these animals were stunned by a blow on the head.

The coronary constricting action of these ergot alkaloids has been ignored in the past. In the light of the present findings it would seem wise to give careful consideration before administering either of them to a patient suspected of having coronary disease.

Oviduct.

The oviducts of 7 hens were used in these experiments. Strips of the uterus, isthmus, and albumen-secreting portion were suspended in oxygenated Ringer-Locke solution and the response to ergonovine, ergotamine, ergotoxine, and epinephrine recorded.

With ergonovine the only consistent results were obtained on the uterine portion, which always showed an increase in tone to concentrations of this drug between 1 : 20,000 and 1 : 14,000. The response was frequently very slight, and with weaker concentrations no effects at all were obtained. The isthmus and albumen-secreting portion were inconsistent and feeble in their reaction to concentrations as high as 1 : 14,000 (fig. 5).

The response to ergotamine was completely erratic. Sometimes the tone and amplitude were increased; in other instances quite the reverse obtained. The drug seemed more powerful than ergonovine, however, for concentrations of 1 : 40,000 were rarely without effect in one direction or the other. Because of the report of McKenney, Essex, and Mann [1932] that all portions of the oviduct were consistently stimulated by ergotoxine at a concentration of approximately 1 : 600,000, this alkaloid was included in the present series of experiments although

there was little expectation that any real qualitative difference between it and ergotamine would be observed. It was found that ergotoxine-ethanesulphonate in concentrations of 1 : 4,000,000 to 1 : 80,000 were quite uniformly without effect on the oviduct, except in one instance in which a concentration of 1 : 80,000 caused a slight inhibition.

Epinephrine 1 : 2,000,000 to 1 : 500,000 elicited much more consistent responses than did the ergot alkaloids. All three portions of the oviduct

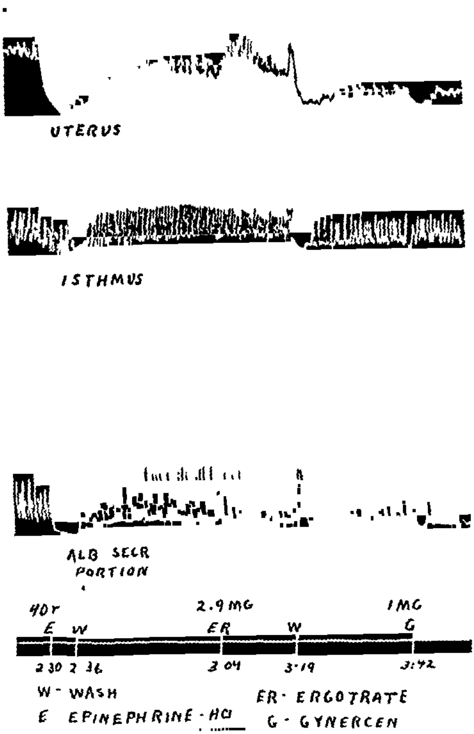

FIG. 5.—Response of the oviduct to epinephrine, ergonovine, and ergotamine. Bath volume 40 c.c.

showed an inhibition of spontaneous contractions and a sudden loss of tone, except in the case of one strip of isthmus in which the tone was increased. These findings again disagree with those of McKenney, Essex, and Mann [1932], who state that the isthmus and albumen-secreting portion respond to epinephrine with a maximal contraction and only the uterus shows a relaxation.

It seems possible that the discrepancies between the findings reported here and those of McKenney, Essex, and Mann [1932] may be attributed to the fact that their birds were in active egg production, whereas such was not the case in the present series although the fowls appeared sexually mature.

Intestine.

Ergonovine caused a decrease in tone of isolated strips of gut but had little effect on the spontaneous contractions. The duodenum appeared most sensitive, being relaxed abruptly by a concentration of 1 : 400,000, and showing little tendency to recover even after washing. A concentration of 1 : 80,000 caused the jejunum to relax fairly abruptly, while this same concentration produced only slight relaxation in the ileum. The spontaneous contractions of the colon were the most vigorous. Ergonovine 1 : 400,000 caused a slight relaxation after the first dose, but had no effect even in much more concentrated solutions upon subsequent administration. On the colon of another chicken it required a concentration of 1 : 80,000 to elicit a slight relaxation.

Ergotamine 1 : 160,000 decreased the tone of the duodenum quite permanently. Its response was less abrupt than that of ergonovine. Concentration as high as 1 : 80,000 on the jejunum and ileum and 1 : 40,000 on the colon were ineffective.

Summary.

1. The practicability of the chicken as a laboratory animal is reaffirmed.

2. The alkaloids ergonovine and ergotamine are about equally effective in their action upon the circulatory system of the chicken as judged by the magnitude of the blood-pressure response, but the effects of ergonovine are more transitory.

3. Much smaller doses of ergonovine are required to produce pressor responses in chickens than in cats or in other laboratory animals.

4. In doses of about 10–20 mg. ergonovine and ergotamine are effective in inhibiting but not reversing the blood-pressure response to epinephrine in the chicken.

5. After moderate doses of ergonovine and ergotamine electrocardiograms show only a slowing of the heart. With excessive doses minor changes in the T-wave are observed.

6. In experiments on the perfused hearts of the fowl, cat, and rabbit small amounts of ergonovine and ergotamine give a striking reduction in the coronary flow. Ergotamine is some ten times more potent than ergonovine in this respect and also more persistent.

7. The response of the oviduct to these alkaloids is irregular except in the case of the uterine portion, which is always stimulated by ergonovine. Epinephrine inhibits all segments.

8. Studies on the isolated chicken intestine show a reduction in tone from ergonovine and ergotamine, the former being more effective. The response to both substances decreases from duodenum to colon.

It is a pleasure to acknowledge the helpful guidance of Professor E. M. K. Geiling throughout this investigation. A portion of the

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DYNAMIC VARIATION IN RESPONSE TO BARBITURATES.

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Two forms of variation in the response to drugs can be distinguished. Firstly, the variation between individuals when the response of any individual remains constant (static variation), and secondly, fluctuations in the response of any particular individual (dynamic variation).

Less attention has been paid to dynamic than to static variation. The former is more difficult to measure because it can only be studied in the case of responses which produce no permanent effect and it is necessary to arrange exactly similar environmental conditions in successive experiments.

Clinical experience shows that an individual, who gives an exceptional response to a drug, usually is constant in this respect. This is true not only in the case of responses that are of abnormal character (true idiosyncrasy), but also in the case of exceptional sensitivity or insensitivity to drugs such as anæsthetics and hypnotics.

There is in addition a certain amount of quantitative evidence from animal experiments. Eichler and Smiatek [1937] measured the constancy of the response of rats to various anæsthetics. A population of animals were given a median narcotic dose of a drug, the positives and negatives were separated and a few days later the test was repeated in the two groups. Technical details were carefully controlled. In the case of rats exposed to chloroform vapour, the authors found the high correlation coefficient (r) of $+0{\cdot}81$ for the individual responses in successive experiments. In similar experiments with avertin the correlation coefficient was $+0{\cdot}61$, whilst with avertin followed by chloroform and chloroform followed by avertin the correlation coefficients were $+0{\cdot}58$ and $+0{\cdot}48$ respectively. On the other hand, with chloroform or avertin followed by the barbiturate eunarcon there was no certain positive correlation.

Kohn [1938] measured the quantities of nembutal and amytal needed to produce respiratory paralysis in rabbits when given by slow intravenous infusion. He found a considerable variation in the susceptibility of the same animal on different occasions but concluded that this

Experiments.

The authors made experiments with the barbiturates sodium phanodorm and sodium evipan in order to determine the constancy of individual responses. Sodium phanodorm (100 mg./kg. intraperitoneal) was given to 73 mice. The surrounding temperature was 30° C. This dose produced hypnosis in 26 animals (36 per cent.). The positive and negative groups were divided and 4 days later the test was repeated. On this occasion hypnosis was produced in 34 mice (48 per cent.). The results were distributed as follows (+ = narcosis and – = no narcosis):—

1st Test.	2nd Test.	
+	+	20
+	–	6
–	–	33
–	+	14

This result shows a correlation coefficient (r) of 0·72, which is similar to the values obtained with narcotics in similar tests by Eichler and Smiatek [1937].

In a second experiment 60 mice were given 132 mg./kg. phanodorm intraperitoneally. Eighteen animals that showed marked depression but did not show full narcosis for as long as 10 min. were discarded and two groups were kept, namely, an insensitive group of 23 mice which showed no narcosis and a sensitive group of 19 mice that were narcotised for more than 10 min. (average duration 40 min.). These two groups were tested a week later with the same dosage. The results were as follows:—

	Insensitives.	Sensitives.
No narcosis	10	1
Narcosis more than 10 min.	6	18
Narcosis 1–10 min.	7	0

In the case of the 24 mice showing more than 10 min. narcosis the average duration was 32 min. Owing to the selection exercised it is not easy to state a figure for the correlation coefficient, but the obvious difference in the behaviour of the two groups confirms the previous conclusion that animals show constancy as regards the dose of barbiturate needed to produce narcosis.

The 6 insensitives and 1 sensitive which showed definitely divergent responses were discarded. Four days later the remaining insensitives and sensitives were given twice the previous dose of phanodorm (264 mg./kg.) and the duration of narcosis of each individual was measured. This experiment was repeated again 3 days later.

variation was not as pronounced as the variation among different animals.

Hemmingsen [1939] carried out cross-over tests with insulin convulsions in mice. He found that by separating the positive and negative groups it was possible to reduce the standard deviation of the responses from 12–25 per cent. of the median to 7–10 per cent. Bliss and Marks [1939] measured the fall of blood sugar produced by insulin in rabbits. They made cross-over experiments and concluded that the differences in overall susceptibility between rabbits exceeded the variation in the responses given by a single animal by more than eight times.

Emmens [1939] made repeated tests of the action of œstrone on a group of mice. He concluded that the relative sensitivity of a mouse was not constant, but that it was less variable than the total range of sensitivity of the group.

These results all indicate a small dynamic variation, but in other cases this has been found to be large.

Sampson and Fernandez [1939] measured the response of rats to a dose of thujon suspension which caused 50 per cent. incidence of convulsions This dose was given 10 times to each of 40 rats. The sensitivity of the group as a whole did not vary and there were 203 convulsive responses out of the total of 400 experiments.

These results can be analysed to estimate whether the rats were constant in their behaviour or whether the incidence of convulsions was a matter of random chance. The simplest treatment is to measure the number of positive results scored by each individual. If the animals were completely consistent in their response there would be 20 scores of 10 and 20 scores of zero. If the responses were completely random they would show a binomial distribution. The results obtained were as follows:—

Positive scores.	0	1	2	3	4	5	6	7	8	9	10	Total.
No. of such scores observed.	1	0	6	4	6	6	6	6	0	3	2	40
No. of scores according to random distribution.	0	0	2	5	8	10	8	5	2	0	0	40

The results indicate that 5 animals were more sensitive than the average and 5 were more resistant, whilst the responses of the remaining 30 followed the distribution to be expected if on each occasion with each individual there was an even chance of a positive or negative response.

The various experiments quoted show therefore very different results as regards the extent of dynamic variation.

were made on pigs 2–6 kg. in weight. These cases can be divided into three nearly equal groups according to the duration of the first narcosis.

Range of duration of first response (in min.).	No. of animals in group.	Average duration in min.	
		1st response.	2nd response.
7–10	9	8	14
10·5–14·5	11	12·5	13
15–22	12	17	14

The results show that the duration of the second narcosis is the same in all cases and therefore provide no evidence for any persistent *individual variation as regards duration* of response.

One of the authors [Clark, 1939] gave a preliminary account of measurements of the duration of evipan narcosis in mice, which showed that there was no obvious correlation between the durations of the responses of single individuals when these were given repeated injections. These results were open to the criticism that the successive experiments were carried out at different room temperatures (20° C. and 40° C.). We therefore made more extensive experiments on this subject. A population of male mice were given 100 mg./kg. sodium evipan intraperitoneally. The animals were kept at 30° C. and the duration of narcosis of each individual was measured. The individuals were marked, and the test was repeated five times at weekly intervals. A population of 40 mice was used, 22 survived the whole test and only the responses of the survivors were considered. The average duration of sleep differed on different days, and a few mice showed exceptionally long durations. These facts and the relatively small numbers make it necessary to use crude methods of analysis.

The responses for each test were classified into three groups; medium responses which were within 5 min. of the average response for the day and the responses shorter and longer than these. The totals of the three groups were of a comparable magnitude (33 short, 30 medium, and 47 long responses). The score of each mouse in each class of response in the five tests was measured. If a mouse were completely consistent in its behaviour then it would fall each time into the same group, and its score would be 5 in one group and 0 in the two other groups. If a mouse were completely variable in its behaviour, the most likely score would be 2, 2, and 1. The variability was estimated by measuring the frequency of the various kinds of scores. The total number of scores was 66 (3 for each of 22 mice) and the possible values ranged from 0 to 5. If the mice were completely

Thirty animals (15 insensitive and 15 sensitive) survived the whole test. The mean durations of narcosis in these animals were as follows:—

	Mean durations of narcosis in min.		
	1st test.	2nd test.	Average.
Sensitive . .	134	157	145
Insensitive . .	94	99	96
Average . . .	114	128	

The mean duration of narcosis of the sensitive group was therefore $1\frac{1}{2}$ times as great as that of the insensitive group.

The distribution of the responses can be summarised as follows (narcosis shorter than the average for the test = –, longer = +):—

1st test.	2nd test.	Sensitive.	Insensitive.
+	+	9	1
+	–	0	2
–	–	3	9
–	+	3	3

The table shows the correlation as regards duration of narcosis in the two tests. In 3 individuals the two responses differ widely, but in 22 cases out of 30 there is not more than 25 min. difference between the two responses.

The figures dealt with relate to small groups of selected animals; the figures in the table also indicate that the variation in the duration of the response is not distributed symmetrically. Hence it is not possible to calculate the correlation coefficient. The results indicate, however, that groups of selected animals show a constant difference as regards duration of action and that in such cases there is a definite correlation between the successive responses given by any individual.

We found, however, that it was not possible to demonstrate any constancy in the durations of the responses to barbiturates in groups of unselected animals. Donald and Raventós [1939] measured the durations of narcosis produced by repeated intravenous injections of sodium evipan (20 mg./kg.) in a group of young pigs. They could not demonstrate any persistent individual variation in susceptibility.

We have examined the protocols of these experiments and find records of 32 cases in which two measurements per individual

were made on pigs 2–6 kg. in weight. These cases can be divided into three nearly equal groups according to the duration of the first narcosis.

Range of duration of first response (in min.).	No. of animals in group.	Average duration in min.	
		1st response.	2nd response.
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The responses for each test were classified into three groups; medium responses which were within 5 min. of the average response for the day and the responses shorter and longer than these. The totals of the three groups were of a comparable magnitude (33 short, 30 medium, and 47 long responses). The score of each mouse in each class of response in the five tests was measured. If a mouse were completely consistent in its behaviour then it would fall each time into the same group, and its score would be 5 in one group and 0 in the two other groups. If a mouse were completely variable in its behaviour, the most likely score would be 2, 2, and 1. The variability was estimated by measuring the frequency of the various kinds of scores. The total number of scores was 66 (3 for each of 22 mice) and the possible values ranged from 0 to 5. If the mice were completely

consistent in their behaviour then values of 0 and of 5 would occur most commonly.

The distribution of scores was as follows:—

Score.	Incidence.
5, 0, 0	0
4, 1, 0	5
3, 2, 0	5
3, 1, 1	4
2, 2, 1	8

No mouse attained complete constancy, and the most random score was the most frequent. This result therefore indicates a lack of constancy in the successive responses of the individuals.

Dr. W. O. Kermack kindly calculated for us the incidence of scores to be expected if the variation in the duration were of a random nature; that is to say if there was no correlation between the character of the responses given by any particular individual.

The results were as follows:—

Value of score.	0	1	2	3	4	5
Distribution of scores.						
(*a*) Calculated for random variation.	9·5	21·4	20·6	10·9	3·2	0·4
(*b*) Found with mice.	10	21	21	9	5	0

The distribution of the scores is therefore nearly the same as that which would occur if the responses were of a random character.

Discussion.

The tests made of the *individual constancy of the responses of mice* to barbiturates showed that when the test measured the intensity of action then the individuals showed a constancy as regards their responses. This agrees with previous work carried out with other narcotics and also is in accordance with clinical experience.

Measurements of the duration of responses to repeated doses of barbiturates showed that when mice were selected so as to obtain sensitive and insensitive groups, then the different groups showed clear differences as regards the average durations of their responses to barbiturates and there was a definite positive correlation between the successive responses of individuals. When, however, the durations of successive responses of an unselected group of animals was measured,

the results failed to demonstrate any correlation between the durations of successive responses. The duration of response is therefore less constant than is the intensity of the response. This variation in duration of response may be due to fluctuations in the rate of destruction of the drug.

Krautwald and Oettel [1937] and Krautwald [1937] measured the daily excretion of barbitone, phenobarbitone, and phanodorm and noctal given to dogs in constant daily doses over prolonged periods. Their protocols show very considerable daily fluctuations in the amounts of drugs excreted, a fact which suggests a considerable fluctuation in the amount of drug daily destroyed in the body.

Dynamic variation is an undesirable character in a response that is used for the purposes of drug standardisation. Our results indicate that from this point of view estimates based on durations of responses are likely to be less reliable than those based on the intensity of responses.

The duration of response to a barbiturate appears to be an attractive method of measuring the intensity of action since it provides a graded measure of the action produced. Moreover, there is a constant relation between dosage and the average duration of the responses of a group of animals.

It has been shown [Raventós, 1938] that the duration of narcosis is dependent on the room temperature, and the facts mentioned in this paper show that it also is influenced by unknown variable factors. Hence the theoretical significance of the duration of response is somewhat uncertain.

TABLE.—DURATIONS EXPRESSED AS MINUTES LONGER OR SHORTER THAN THE MEAN.

	−75	−50	−25	−0	+25	+50	+75	+100	1st test.
−75									
	1	..	..	..	..	..	..		1
−50									
	2	6	..	1	..	1	..		10
−25									
	..	2	1	1	2	..	1		7
0									
	..	..	1	1	1	..	..		3
+25									
	..	..	..	1	2	1	1		5
+50									
	1	..	..	1	1	..	1		4
+75									
2nd test.	4	8	2	5	6	2	3		30

Conclusions.

1. There is a fairly high individual constancy as regards sensitivity to barbiturates; individuals which are narcosised by a median hypnotic dose are likely to show the same response when the test is repeated.

2. There is less individual constancy as regards the duration of narcosis produced by barbiturates. Such constancy can be demonstrated in groups of mice selected as regards sensitivity, but in unselected groups we could not demonstrate any constancy.

The expenses of this research were defrayed by a grant from Messrs. Imperial Chemical Industries (Dyestuffs Group), for which the authors express their thanks.

We also thank Dr. W. O. Kermack for his kind assistance in the mathematical analysis of the results.

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THE SITE OF ACTION OF THYROXIN ON WATER-METABOLISM.

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That "spontaneous" and post-operative thyroid deficiency are accompanied by oliguria, water-retention and hyper-proteinæmia, while clinical and experimental hyperthyroidism produce the opposite states, are equally notorious facts.

Alleging that thyroid extract fails to produce an increase in kidney volume and that no proof of its action on the kidney was otherwise forthcoming, Eppinger [1917] envisaged in myxœdema a retardation in the katabolism of tissue proteins and a consequent increase in their capacity to retain water. An extra-renal action of thyroid has since been emphasised by several investigators. Ellinger [1922] laid stress on the influence of thyroxin on the swelling of plasma and tissue colloids, and was led to regard this substance as the prototype of diuretics with a primary extra-renal action. Asher [1920], Meyer-Bisch [1923], and Fujimaki and Hildebrandt [1924] developed the thesis of an extra-renal action of thyroid by studying its action on general metabolism, on the formation of œdema fluid and on the state of hydræmia. Moreover, Epstein [1929], from an investigation of the conflict of action of thyroxin and several narcotic substances on water-diuresis, favoured the exclusion of a renal component from the polyuric effect of the hormone. More recently Marx [1935] concludes that no direct action of thyroxin on the kidney has been demonstrated, and he himself failed to observe any diuretic effect from this substance in the heart-lung-kidney preparation. Meanwhile, however, van Creveld [1922] has subjected Eppinger's theory of œdema formation to severe criticism, and has shown that thyroid extract produces renal vaso-dilatation in the perfused kidney of the frog. Up to the present, and so far as we are aware, no direct evidence has been forthcoming which allows the exclusion of a direct renal action of thyroid extract or of thyroxin in the mammal.

The object of this paper is to present experiments which give an unequivocal demonstration of the localisation in the kidney of the polyuric action of thyroxin in the mammal.

Conclusions.

1. There is a fairly high individual constancy as regards sensitivity to barbiturates; individuals which are narcosised by a median hypnotic dose are likely to show the same response when the test is repeated.

2. There is less individual constancy as regards the duration of narcosis produced by barbiturates. Such constancy can be demonstrated in groups of mice selected as regards sensitivity, but in unselected groups we could not demonstrate any constancy.

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diuresis was producible, the kidneys from the thyroxinised animal should give an earlier and stronger response than those from the control, were there a direct renal action of thyroxin. Now the implantation of kidneys into an anæsthetised donor is associated with conditions under which water-diuresis does not occur. It was shown earlier, however [Brull, 1938], that anæsthesia of at least the transplanted kidneys can be avoided by taking them from an animal killed

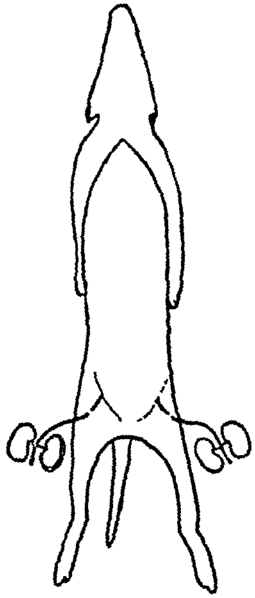

FIG. 1.—To illustrate the method used in the perfusion of two pairs of kidneys by one dog. The perfusing animal is anæsthetised with chloralosane, and the kidneys are taken from unanæsthetised dogs shot immediately before the transplantation of their kidneys. One pair of kidneys is taken from a normal, the other from a thyroxinised animal. The arterial connections only are represented.

immediately before the transfer, a transfer which can be performed under these conditions without stopping the circulation for more than five minutes. Moreover, if the anæsthetic (chloralose) is injected into the donor at least half an hour before the kidneys are anastomosed with its circulation, these kidneys behave like unanæsthetised organs. Fig. 1 shows schematically the way in which the kidneys are connected with the donor. The problem now resolved itself into the production, in such a preparation, of water-diuresis by some means other than by the intestinal absorption of water. Verney's papers again provided the necessary suggestion. According to to this author [Verney, 1936] water-diuresis is preceded by and dependent upon inhibition of secretion from the posterior lobe of the pituitary. If this be true, removal of the posterior lobe of the pituitary should be followed by a polyuria similar in type to water-diuresis in the normal animal. Verney [1926],

Choice of Method.

In 1926 Verney demonstrated the liberation of an antidiuretic substance from the pituitary body into the blood-stream. Compère [1933] confirmed in our laboratory this fundamental finding by using our method of simultaneously implanting the kidneys of one dog into two others ("donors") [Brull, 1933]. In this preparation the kidneys, after anastomosis with the circulatory systems of two donors through the medium of the aortic and inferior caval segments carrying respectively the origins of the renal arteries and the embouchements of the renal veins, can be switched from the one donor to the other and *vice versa* without any interference with their circulation. In Compère's experiments the one donor was polyuric as a consequence of the removal of its pituitary, the other served as control; and Compère was able to show that every time the anastomosed kidneys were switched from the control donor to the polyuric one the anastomosed kidneys themselves became polyuric and *vice versa*. Verney's experiments [1926], confirmed by Compère's work [1933], furnished the first experimentally induced example of an hormonal influence on the processes in the kidney whereby the regulation of the composition of the "milieu intérieur" is implemented.

Again, that parathyroidectomy leads to a fall in the excretion of phosphate by the kidney has been known for many years. After Collip [1925] had isolated an active extract from the gland it was shown that injection of this extract produced a rise in phosphate excretion [Albright, Baur, Ropes and Aub, 1929]. Although suggestions appeared to the effect that the parathyroid regulated phosphorus excretion by an action of its secretion on the kidney, no direct proof of this was forthcoming until we succeeded, by means of several techniques of cross circulation [Brull, 1936, 1939], in demonstrating the renal localisation of the influence of this gland. This work furnished a second example of the active participation of internal secretions in catalysing or inhibiting secretory processes in the kidney, and we were thereby encouraged to investigate the site of action of thyroid by similar methods.

In their paper on water-diuresis, Klisiecki, Pickford, Rothschild and Verney [1933] give suggestive curves showing that in thyroxinised dogs water-diuresis starts earlier and reaches a higher maximum than in normal animals. The authors write: "The simplest interpretation of our results would appear to be in terms of a direct effect of thyroxin in the kidney, since the outstanding characteristic of its action is an increase in the maximum rate of secretion." One felt that if one could realise an experiment in which two pairs of kidneys were fed by the same blood, one pair originating from a normal control and the other from a thyroxinised animal, an experiment, moreover, in which water-

diuresis was producible, the kidneys from the thyroxinised animal should give an earlier and stronger response than those from the control, were there a direct renal action of thyroxin. Now the implantation of kidneys into an anæsthetised donor is associated with conditions under which water-diuresis does not occur. It was shown earlier, however [Brull, 1938], that anæsthesia of at least the transplanted kidneys can be avoided by taking them from an animal killed

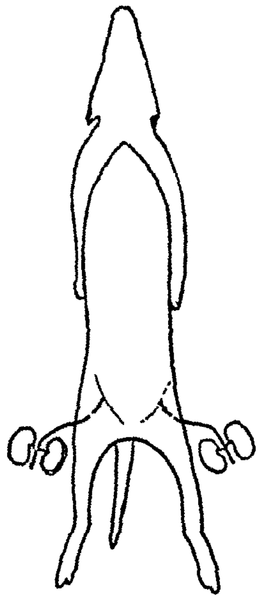

Fig. 1.—To illustrate the method used in the perfusion of two pairs of kidneys by one dog. The perfusing animal is anæsthetised with chloralosane, and the kidneys are taken from unanæsthetised dogs shot immediately before the transplantation of their kidneys. One pair of kidneys is taken from a normal, the other from a thyroxinised animal. The arterial connections only are represented.

immediately before the transfer, a transfer which can be performed under these conditions without stopping the circulation for more than five minutes. Moreover, if the anæsthetic (chloralose) is injected into the donor at least half an hour before the kidneys are anastomosed with its circulation, these kidneys behave like unanæsthetised organs. Fig. 1 shows schematically the way in which the kidneys are connected with the donor. The problem now resolved itself into the production, in such a preparation, of water-diuresis by some means other than by the intestinal absorption of water. Verney's papers again provided the necessary suggestion. According to to this author [Verney, 1936] water-diuresis is preceded by and dependent upon inhibition of secretion from the posterior lobe of the pituitary. If this be true, removal of the posterior lobe of the pituitary should be followed by a polyuria similar in type to water-diuresis in the normal animal. Verney [1926],

indeed, has shown that even under chloralose anæsthesia a dog may become polyuric after removal of its pituitary gland, and this fact has been confirmed in our laboratory [Brull, 1930; Compère, 1933]. We have found that when a dog under chloralose receives a constant intravenous infusion of saline, the rate of urine secretion increases only a little, and that no polyuria occurs unless the pituitary has been previously removed.

Using, then, the preparation described above—a dog with two pairs of kidneys anastomosed with its femoral vessels, the one pair being taken from a normal, the other from a thyroxinised animal—we proceed to hydrate the donor by a constant infusion of normal saline, and later to remove the pituitary [1] in order to produce a polyuria. If thyroxin has indeed a direct renal action, we should expect a polyuria earlier in onset and greater in degree from the thyroxinised than from the control kidneys.

Results.

Experiment I.—A bitch weighing 24 kg. receives 0·12 g. chloralosane per kg. i.v. The two femoral arteries and veins are dissected and Payr's cannulæ attached, so that the vessels are ready to receive the pairs of kidneys from the two other animals. One of these, a dog weighing 8 kg. is the normal control, the other, weighing 9 kg., has received 1 mg. thyroxin subcutaneously on each of the three days before the experiment and a further dose of 1 mg. immediately before the experiment began (total dose of thyroxin 0·44 mg./kg. over four days). The two kidney-dogs are killed by a shot through the skull, the kidneys immediately removed with the interlying segments of aorta and vena cava, and these segments anastomosed with the femoral vessels of the donor. The anastomosis of the control and of the thyroxinised kidneys is accomplished 55 and 70 min. respectively after the anæsthetic has been given to the donor. The weight of the kidney pairs was 46 and 54 g. respectively. The arterial pressure is measured at the free end of the aortic segment supplying one pair of kidneys, and a constant and fast infusion of normal saline (30 c.c./min.) is given through the vena cava of the other pair. The responses of the two pairs of kidneys are shown in fig. 2 (c.c. urine per hour per gram of fresh kidney). As will be seen, the water outputs of the two pairs of kidneys ran closely parallel and increased about sevenfold as the result of the profound hydræmia: the plasma proteins fell from 5·0 to 2·2 g./100 c.c. during the period of infusion. Clearly no difference is appreciable between the responses of the kidneys from a normal dog and of those from a dog thyroxinised to this degree, viz. 0·44 mg./kg. body-weight.

Experiment II.—We then decided to increase the dose of thyroxin. The donor weighed 17·2 kg., the kidney-dogs 5·8 kg. (control) and

[1] In our experiments the pituitary has been removed by the temporal route.

6·15 kg. (thyroxinised), the thyroxinised animal having received a total dose of 1 mg. thyroxin/kg. over the six days preceding the experiment. The thyroxinised dog lost 0·45 kg. in weight during this period.

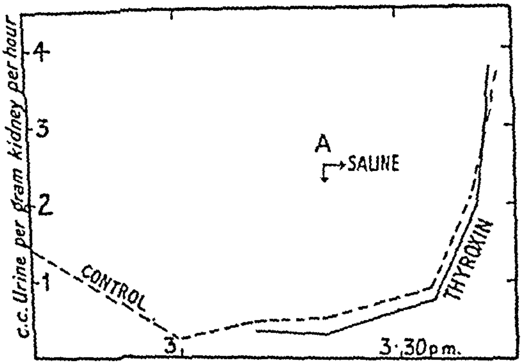

FIG. 2.—Urine outputs of two pairs of transplanted kidneys, the one (– – –) from the kidneys of a normal dog, the other (——) from those of a dog previously injected with thyroxin (0·44 mg./kg. body-weight). At A a fast infusion of saline (30 c.c./min.) was begun: this was continued throughout the experiment. Ordinates : c.c. urine/g. fresh kidney tissue/hour. Abscissæ: time in hours.

The technique in this experiment was the same as in Experiment I., with the exception that saline was infused at a much slower rate, viz. 4 c.c./min. The weight of the control kidneys was 28·5 g., that

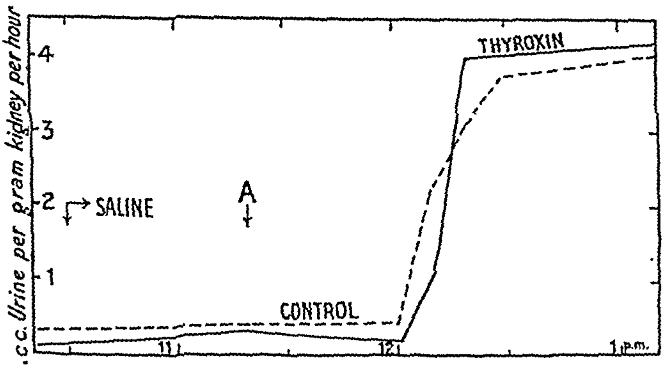

FIG. 3.—Same technique as before (see fig. 2). Dose of thyroxin 1 mg./kg. body-weight. The saline was infused at a slow rate (4 c.c./min.). At A the pituitary was removed. Ordinates and abscissæ as in fig. 2 (*q.v.*).

of the thyroxinised 27·5 g. The resultant hydræmia was small, the plasma proteins falling from 5·4 to 4·6 g./100 c.c. during the period of infusion. The results of this experiment are shown in fig. 3. As will be seen, the saline infusion produced a relatively greater increase in urine flow from the thyroxinised kidneys, but the effect was small and no polyuria from either pair supervened. Forty-five minutes after hypophysectomy, however, polyuria appeared, but the course of urine

flow from the thyroxinised kidneys ran closely parallel with that from the control pair. In a further and similarly conducted experiment, however (Experiment III., fig. 4), removal of the pituitary was followed by a polyuria of earlier onset and much greater degree from the thyroxinised than from the control pair of kidneys. The rate of saline infusion was 4 c.c./min.: the weight of the control kidneys 28 g. and of the thyroxinised 24 g. This result encouraged us to investigate the

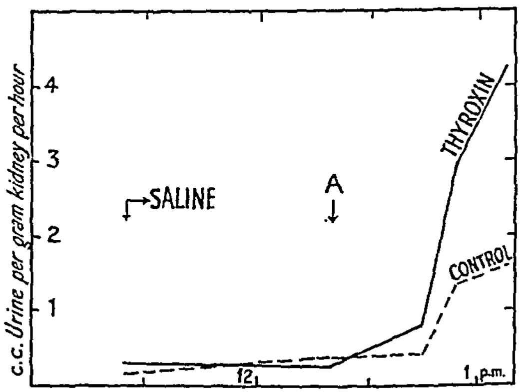

Fig. 4.—Same technique as before (see fig. 2). Dose of thyroxin 0·9 mg./kg. body-weight. Slow infusion of saline. At A the pituitary was removed. Ordinates and abscissæ as in fig. 2 (*q.v.*).

effects of a further increase in the dose of thyroxin and three experiments will now be reported in which larger doses than hitherto have been employed.

Experiment IV.—In this experiment the donor weighed 20 kg., the control kidney-dog 7·7 kg. and the thyroxinised 6·6 kg. The control kidney-pair weighed 35 g., the thyroxinised 71 g. The technique and rate of saline infusion were the same as in the two previous experiments, but 4·5 mg. thyroxin/kg. were given over the 9 immediately preceding days. The results of this experiment are shown in fig. 5. Even before the slow infusion of saline was begun, the water-output of the transplanted thyroxinised kidneys was about double that of the control pair. Moreover, while the control kidneys gave no response, or a minimal only, to saline infusion even after two hours, the thyroxinised kidneys became markedly polyuric after one hour's infusion only. A fall in the donor's arterial pressure temporarily reduced this polyuria, and after the pituitary was removed a polyuric response by both the thyroxinised and the control kidneys appeared, but the response by the thyroxinised pair was greater absolutely than that by the control pair. This experiment confirms the findings of the previous one, and shows, in addition, that sufficiently thyroxinised kidneys give a larger response than the control organs not only to hypophysectomy but to hydræmia as well.

Experiment V.—Here the same technique was adopted with the same rate of saline infusion, the dose of thyroxin in this experiment being 5 mg./kg. given over a period of 9 days. The donor weighed 22·2 kg., the control kidney-dog 7 kg., and the thyroxinised 5·3 kg. The control kidneys weighed 37·5 g., the thyroxinised 30·0 g. The

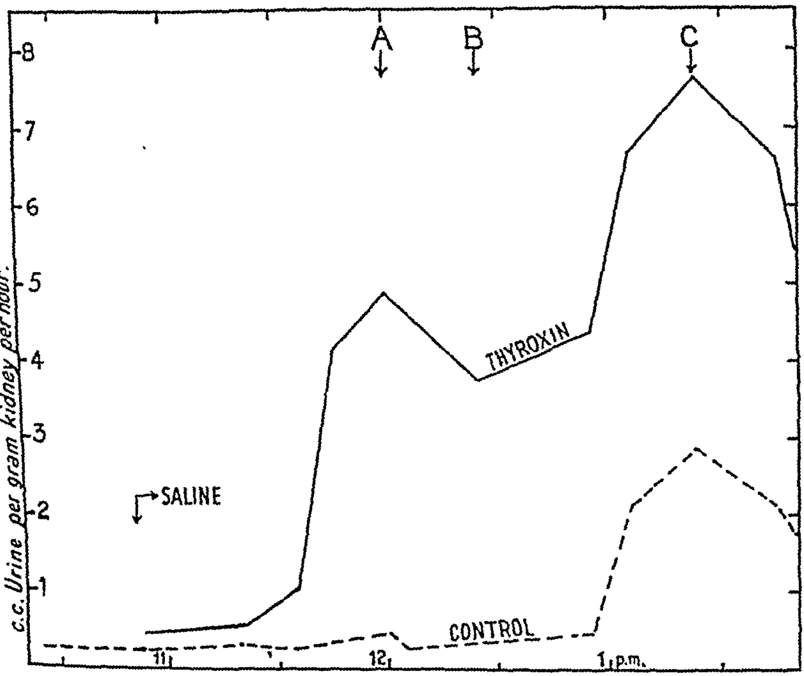

Fig. 5.—Same technique as before (see fig. 2). Dose of thyroxin 4·5 mg./kg. body-weight. Slow infusion of saline. Even before the infusion was started the kidneys from the thyroxinised animal were secreting more rapidly than those from the control, and the difference in rate was accentuated by the infusion. At A the arterial pressure fell from 160 to 120 mm.kg. At B the pituitary was removed. At C there was a further fall in arterial pressure. Ordinates and abscissæ as in fig. 2 (*q.v.*).

results are shown in fig. 6. Here, again, the initial water-output of the thyroxinised kidneys was much higher than that of the control pair, and the response to saline infusion also was greater. A fall in the donor's arterial pressure (from 160 to 115 mm. Hg) suppressed the polyuria of the thyroxinised kidneys, but after hypophysectomy polyuria of even greater magnitude appeared. Unfortunately clotting occurred in the arterial supply to the control kidneys, so that their response to hypophysectomy could not be followed: the polyuric response of the thyroxinised kidneys, however, was much greater than any response we have encountered in control kidneys under these conditions.

Experiment VI.—In this experiment 3·2 mg. thyroxin/kg. was administered over the 3 days immediately preceding. The donor weighed 19 kg., the control kidney-dog 5·6 kg. (one kidney 18·5 g.),

and the thyroxinised 5 kg. (one kidney 14 g.). The technique was the same as that of the last experiment with the exception that no infusion

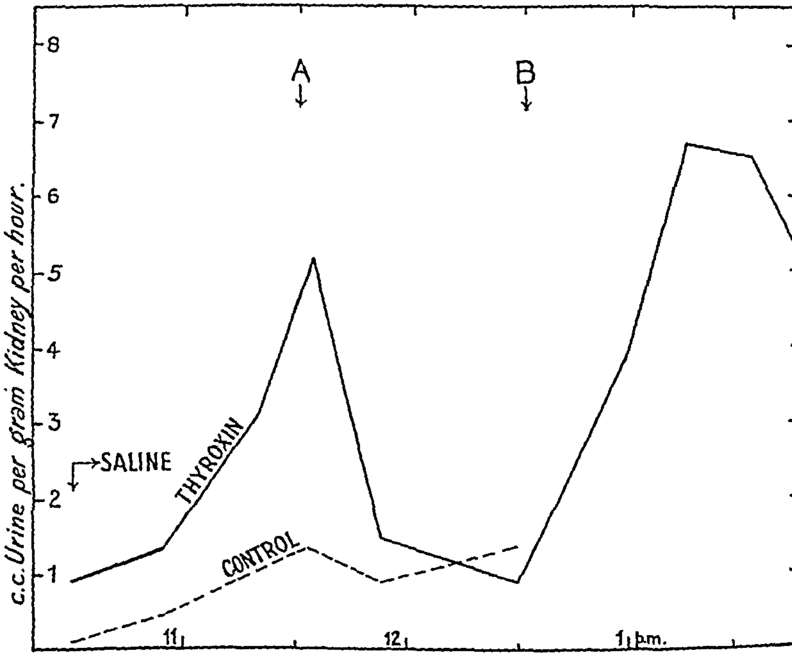

Fig. 6.—Same technique as before (see fig. 2). Dose of thyroxin 4·5 mg./kg. body-weight. Slow infusion of saline. The resting rate of urine secretion by the thyroxinised kidneys is greater than that by the control pair. At A the arterial pressure fell from 160 to 115 mm./Hg. At B the pituitary was removed. Ordinates and abscissæ as in fig. 2 (*q.v.*).

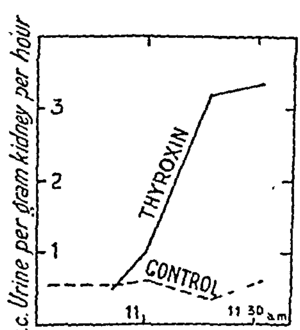

Fig. 7.—Same technique as before (see fig. 2). Dose of thyroxin 3·2 mg./kg. body-weight. No infusion of saline. The urine flow of the thyroxinised kidney steadily increases, that of the control remains constant. Ordinates and abscissæ as in fig. 2 (*q.v.*).

of saline was given. One control and one thyroxinised kidney only were transplanted. The results of this experiment (fig. 7) emphasise the fact previously encountered, namely, that the basal rate of urine

flow from transplanted thyroxinised kidneys is higher than that from the controls. In all our experiments with the higher doses of thyroxin (over 1 mg.), the urine flow of the thyroxinised whole animals was higher than that of the controls, and the experiments reported above show that this polyuria persists even after transplantation and in the absence of any agency or procedure calculated to produce diuresis. In the experiment immediately before us the water-output of the thyroxinised kidney was seven times greater than that of the control.

Discussion.

The method we have used places two pairs of transplanted kidneys under exactly the same conditions. Both are totally denervated, are given no anæsthetic before transplantation, and are perfused at the same arterial pressure and with blood of the same composition by a donor anæsthetised with chloralosane.

Under such conditions, kidneys originating from animals injected with thyroxin in sufficient amount (about 1 mg./kg. body-weight, or more) behave differently from controls. When large doses (3 to 4 mg./kg. body-weight) are administered before transplantation, the water-output after transplantation is larger than that of the controls even in the absence of any stimulation of diuresis. This disparity in response becomes greatly intensified when saline is infused into the donor, and the polyuria following hypophysectomy is likewise greater with kidneys transplanted from a thyroxinised dog than with those transplanted from a normal control. This increased response to hypophysectomy may be seen after doses of thyroxin as low as 0·9 mg./kg. These last facts, in confirming the hypothesis of Klisiecki *et al.* [1933], cited above, namely, that thyroxin affects water-diuresis in the normal animal by acting directly on the kidney, render untenable the views of Eppinger [1917], Ellinger [1922], Marx [1935] and others who consider the diuretic effect of thyroxin as being purely extrarenal. Our experiments, indeed, while not excluding an extrarenal component from the diuretic action of this drug, afford an unequivocal demonstration of a directly induced renal effect.

Summary.

1. Kidneys originating from thyroxinised dogs, when transplanted simultaneously with control kidneys to a donor anæsthetised with chloralosane, have been found to secrete more rapidly and to give larger responses to saline infusion and to hypophysectomy than the controls.

2. The method adopted and the results obtained demonstrate that the diuretic effect of thyroxin is due, at least in part, to a direct action on the kidney.

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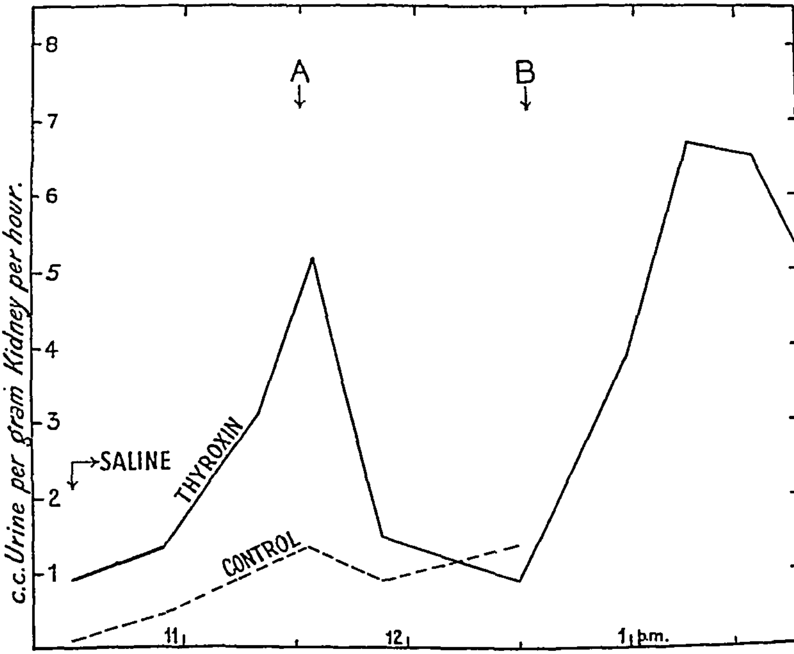

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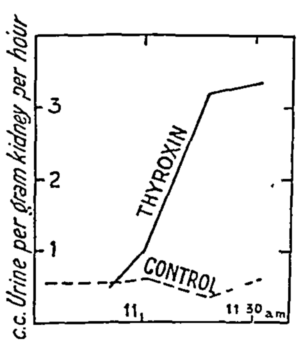

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THE SITE OF ACIDIFICATION OF URINE IN THE FROG'S AND RAT'S KIDNEY. By P. Ellinger. From the Lister Institute, London.

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In spite of the important rôle played by the kidney in the maintenance of the acid-base equilibrium of the body by eliminating a more or less acid urine, the mechanism, and especially the site of the acidification of urine, is still a matter of discussion. There seems to be general consent that the preliminary urine filtered off into the glomerular capsule is alkaline, of the same pH as the blood plasma. This was observed in the living frog by three different investigators using three different methods: microscopically, using phenol-red as indicator, by Bieter and Hirschfelder [1922, 1924]; by estimation of the pH of the glomerular punctate, by Wearn and Richards [1924]; and with the intravital microscope, using the tint of the fluorescence of fluorescein as indicator, by Ellinger and Hirt [1929 *b*, 1930 *a*]. The experiments were confirmed by Montgomery [1935] and by Ellinger [1934 *a*, 1940] and extended with similar results to the glomerular filtrate of the rat's kidney by Ellinger [1934 *b*, 1940]. In the experiments of Ellinger and Hirt [1929 *b*] it was further observed that in German *R. esculenta* in summer the preliminary urine was acidified to a certain degree in the proximal tubules and became still more acid during its passage through the lower urinary tract, especially in the distal tubules. In the same species in winter, urine was found to be far less acid [Ellinger and Hirt, 1930 *a*]. No acidification occurred in the proximal tubules and only slight acidification in the distal and collecting tubules. At the time when this work was done photography of the intravital microscopic pictures was not possible, as photographic emulsions were too inefficient as regards the speed and sensitiveness in the yellow and green. Ellinger and Hirt [1929 *b*, 1930 *a*] had, therefore, to rely on visual observations; and the colour tints of the intravital microscopic pictures could only be reproduced by colour drawings made with a camera lucida. Almost simultaneously Richards [1929] reported that phenol-red introduced directly into the lumen of the frog's tubule changed from red to yellow only in the distal third of the tubules.

In 1934–1937 Ellinger [1934 *a*, *b*, 1940] repeated the intravital microscopic investigations in this country at different seasons of the year with Hungarian *R. esculenta*, and extended the observations to

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hemp, bran, cabbage, and milk, daily; meat twice and fish once, weekly. They were anæsthetised for the experiment by intraperitoneal injection of 30 mg. of chloralhydrate per 100 g. The kidneys were exposed according to the methods described in detail by Ellinger and Hirt [1929 *a*] and by Ellinger [1940]. In rats glomeruli and distal tubules were brought to the surface by taking a shaving from the surface of the kidney in a preliminary operation carried out under ether anæsthesia.

Fluorescein was injected in 1 per mille solution in Ringer's solution into a leg lymph sac in the frog experiments and intraperitoneally into rats. Fluorescein has a very wide and suitable range as an indicator of pH in body fluids. The tint of its fluorescence changes gradually from deep green at pH 9·0 to a brilliant canary yellow at pH 3·0 [Ellinger and Hirt, 1930 *b*]. The colour change can easily be investigated at concentrations of 1/10,000,000; and owing to its luminescence it can be observed in tissues very distinctly, even in very small patches.

In order to render the urine of frogs more alkaline, up to 20 ml. of a one per cent. sodium carbonate solution was injected into the stomach by a stomach-tube in portions of 2–3 ml. during the 24 hours preceding an experiment, or in smaller doses in the course of an experiment. Occasionally this procedure was supplemented by an injection of up to 10 ml. of a one per cent. sodium bicarbonate solution into a leg lymph sac.

In order to obtain a more acid urine some of the rats received by stomach-tube up to 1·0 g. of ammonium chloride in a little water daily for two to three days preceding the experiment.

In frogs all injections were made into the lymph sac of the right leg while the left kidneys were examined. In rats, however, either of the kidneys was used for observation, and the injections were made into the peritoneal cavity.

The kidneys were exposed and an appropriate region was focussed with the objective. The image was illuminated either by the spontaneous fluorescence of the tissues [Ellinger, 1938] or by the white or blue rays from the light source reflected from the superficial layers of the tissues. Then 0·5–1 ml. of the fluorescein solution was injected, and the appearance of the dye and the tint of its fluorescence were followed visually in the various parts of the kidney. From this tint the pH of the fluid in the kidney cells and in the different portions of the lumen of the urinary tract was determined. Colour photomicrographs of the various renal regions were taken at intervals.

Photomicrography of intravital microscopic pictures in black and white has been possible since 1933 [Franke and Sylla, 1933]. During the last two years colour films, particularly for small-size cameras, have been improved so much in speed and sensitivity that correct colour images can be obtained from fluorescence microscopic, and even from intravital microscopic, pictures [*cf.* Hirt, 1939; Ellinger, 1939].

rats. The distinct seasonal differences in the function of the frog's kidney observed in Heidelberg were not seen in this country; the degree of acidification of the urine at all seasons was intermediate between that observed in summer and winter respectively in Heidelberg. The site of acidification was, however, the same as that noted in summer frogs in Heidelberg; some occurred in the proximal, and more in the distal, tubules. In rats there was little acidification in the proximal, considerably more in the distal, tubules. After the injection of an acid salt solution, the excretion of acid urine through the epithelium of the proximal tubules was observed in both frog and rat.

Montgomery and Pierce [1935, 1937] published observations on the site of acidification of urine in *Necturus maculosus* and American *Rana pipiens*. In *Necturus* the urine was taken from different parts of the urinary tract according to the methods of Richards and Walker [1937]; and the pH was estimated either by an indicator method devised by Montgomery [1935] or with a quinhydrone microelectrode devised by Pierce and Montgomery [1935]. There was generally not much acidification of the bladder urine; only one value below pH 6·0 was reported, and this acidification occurred exclusively in a certain segment of the distal tubules. In *R. pipiens* the pH of the content of the urinary tract was estimated by the tint of phenol-red, injected either into a lymph sac or into the glomerular capsule. By this method the site of acidification was found to be restricted to a narrow region of the middle part of the distal tubules.

Although Montgomery and Pierce did not repeat the experiments of Ellinger and Hirt with the same method and the same species of frogs, they stressed the difference between their own results and those of Ellinger and Hirt. This difference was even more emphasised by Richards [1938] in his Croonian Lecture.

In order to clear up the cause of these contradictory results it was decided to repeat the experiments with an improved method in which the subjective visual observation of the changes in colour tints were supplemented by objective records obtained by colour photomicrography.

Methods.

The experiments were performed with English *Rana temporaria* of both sexes in May, June, July, and August, and with rats of both sexes from the Lister Institute's stock. The examination was carried out with the intravital microscope [Ellinger and Hirt, 1929 *a*, 1930 *b*].

Frogs recently captured and kept cold in the dark with access to running water but without food, weighing about 20–30 g., were prepared for the microscopic investigation by percutaneous urethane anæsthesia. Rats weighing 100–200 g. were kept on a mixed diet: oats, wheat,

Results.

Experiments on Frogs.

The results obtained on untreated frogs (8 experiments) are very similar to those obtained by Ellinger and Hirt [1929 *a*] on summer frogs (German *Rana esculenta*) in Heidelberg, and by Ellinger [1934 *a*, 1940] on Hungarian *Rana esculenta* in this country during all seasons. Only a certain proportion of the glomeruli were active, and changes in activity of the glomeruli were not frequent. There was, however, a considerable change in the activity of the various intraglomerular capillaries. Spontaneous fluorescent lyochromes [Ellinger, 1938] were plentiful in the epithelial cells of the proximal tubules as well as in the capsules of active glomeruli and in the lumen of tubules derived from them.

Fluorescein was seen 1–2 min. after its injection into the leg lymph sac in the renal arterioles, where it fluoresced with a distinct greenish tint corresponding to pH 7·5–7·0.[1] Immediately afterwards the capsules of the active glomeruli became brighter with the same greenish tint (pH 7·5–7·0) (*cf.* fig. 1). About 2–4 min. after the injection the lumen of the proximal tubules became bright with a yellowish tint (about pH 6·0), and from these the dye penetrated into the epithelial cells, which fluoresced with greenish yellow light (about pH 6·5) (*cf.* fig. 2). The dye was more concentrated in the lumen of the proximal tubules than in the glomerular capsules, and became even more so when, about 5–7 min. after injection, it appeared in the lumen of the distal tubules with an intense yellow fluorescence (about pH 5·5), while their epithelial cells took up little stain with a greenish-yellow fluorescence (about pH 6·5–6·0) (*cf.* fig. 1). Seven to fifteen minutes after the injection the lumen of the collecting tubules and ureter fluoresced brightly with yellow light (pH 5·5–5·0). About half an hour after the injection, or later, the nuclei of the epithelial cells were stained in the collecting tubules, and some of the dye was separated in larger aggregates in their cytoplasm. The changes were observed only in tubules derived from active glomeruli, those connected with inactive glomeruli remaining unstained. The picture described lasted about 1–3 hr., depending on the amount of fluorescein injected (0·5–1 mg.) and on individual differences in the kidney function. The staining of the epithelial cells of the collecting tubules, however, remained unchanged for 10 hr. and more. The photomicrographs 1 and 2 were taken when the elimination of fluorescein of the kidney was at its height, about 20 min. after its injection.

[1] Small individual variations in the pH of the corresponding parts of the kidneys were observed in animals treated in the same manner before or during the experiment. The data given represent the extreme values found for the pH of the particular tissue, etc.

Of the various colour films available in the summer 1939, Kodachrome Type A proved to be the most suitable for the purpose. It requires only short exposure and is highly sensitive to yellow and green rays, the predominant colours in fluorescein fluorescence. For the true reproduction of the original colour tint accurate exposure is essential. Determination of the latter is difficult, since at present no photometer exists which permits measurement of the light intensity of a fluorescence microscopic picture, and visual estimation is likely to involve errors owing to the heavy contrasts in the image. Under certain circumstances this difficulty can be overcome by the aid of a black and white film of the same colour sensitivity and of known speed; the exposure for the colour film is then adjusted in proportion to the relative speeds of the two films. Panatomic X Film (Kodak) was used for this purpose. This procedure is suitable only if little or slow change occurs in the microscopic image. In studying the function of a living organ, however, the picture and the intensity of the illumination change continually, and one has to rely on visual estimation of the light intensity. It is then advisable to make three or four exposures of the same microscopic picture in the ratios of one, two, four, eight.

The colour film is far more sensitive to ultraviolet and short violet rays than to those of longer wave-lengths, and even traces of ultraviolet rays will spoil entirely the proper reproduction of the colours. It is therefore essential to exclude the slightest trace of the primary source of light which might be reflected back from the surface of the object from passing into the camera. This can be achieved by the use of adequate trap filters of sufficient thickness. When choosing the filters the spectral sensitivity of the emulsion must be taken into account. (Filter GG 11, 2–3 mm. thick, Jenaer Glaswerk Schott und Gen., has proved suitable; it is impermeable to all rays shorter than 450 $m\mu$, and fully permeable for all rays longer than 500 $m\mu$.)

Great care has to be taken in fixing the object sufficiently without interfering with its function and its blood-supply. Frequently it is extremely difficult to avoid slight movements of the object caused by pulsation of the heart or respiratory movements of the lungs which make photomicrography impossible; sometimes even the pulse-wave in the arteries of the organ may spoil the photograph. Since the surface of the object is generally not plane, and any procedure to render it plane may spoil its function and is therefore to be avoided, often only one portion of the photograph can be focussed accurately.

It might be stressed that in intravital microscopy photomicrography cannot replace visual observation by which different levels may be examined in such rapid succession that they provide a three-dimensional image. The value of colour photomicrography, however, is that it provides an objective record of the colour tints of the object.

Fluorescein injected into the peritoneal cavity was seen almost immediately in the renal capillaries and nearly simultaneously in the capsules of active glomeruli with the same green fluorescence (pH 7·5–7·0) (fig. 4). Very soon afterwards, the dye appeared in the lumen and epithelium of the proximal tubules. The colour of the fluorescence in both lumen and epithelium varied from green (pH about 7·0) to a slightly yellowish green (pH about 6·5–6·2) (fig. 5). The former tint was observed when the pH of the bladder urine was above 6·0, the latter when the bladder urine was more acid. This acidity varied individually, and seemed to depend on metabolism and diet; slightly acid bladder urine of pH around 6·5 was most commonly observed. The dye was slightly more concentrated in the lumen of the proximal tubules than in the glomerular capsules, and was still more so in the lumen of the distal tubules, where it appeared about 2–5 min. after the injection. The colour of the lumen of the distal tubules indicated to a pH of between 6·5 and 5·5; and the same tint was observed, but more faintly, in their epithelium (fig. 4). This staining began to fade in 30 min. to 3 hr. after the injection according to the dose of fluorescein (0·5–1 mg.). The staining disappeared from the glomeruli, the lumen of the proximal and of the distal tubules, the epithelium of the distal and of the proximal in this order.

Three rats which had been given ammonium chloride for two to three days showed under the microscope a slightly decreased number of active glomeruli in comparison with untreated rats, but the spontaneous fluorescence of lyochromes appeared unchanged. When fluorescein was injected into the peritoneal cavity of such rats no marked differences could be observed in the time of the staining of the various parts of the kidney and of the elimination of the dye. The tint of the fluorescence in the blood capillaries and in the glomerular capsules was the same as in normal rats (pH 7·5–7·0); but in the lumen and the epithelium of the proximal tubules a more yellow tint was observed (pH 6·5–6·0) (fig. 6) and a far brighter and more brilliant yellow colour (pH 5·5–5·0) was seen in the lumen and epithelium of the distal tubules. After 1–2 hr. the staining disappeared in the same order as in untreated rats.

Discussion.

The results described above and the colour photomicrographs (figs. 1 and 2) show that in normal summer frogs (English *Rana temporaria*) after the injection of fluorescein the preliminary urine fluoresces with a green tint in the glomerular capsules, with a greenish-yellow tint in the lumen of the proximal tubules, and with a yellow tint in the lumen of the distal and collecting tubules and ureter. After either an acute or a more prolonged administration of alkali the colour of the fluorescence changes into a green tint in the lumen of the proximal

Three frogs were treated with sodium carbonate (up to 5 ml. one per cent.) and sodium bicarbonate (up to 5 ml. one per cent.) solution, when the elimination of fluorescein was at its height. The fluorescence in the lumen and epithelium of the proximal tubules became gradually more greenish in colour, the change being more pronounced in the lumen than in the epithelium. After 30–40 min. the tint in the lumen was the same as that in the glomerular capsule (pH 7·5–7·0) (fig. 3). The change of colour was, however, so gradual that it was impossible to decide whether it occurred earlier in the epithelium or in the lumen. The tint of the fluorescence in the lumen of the distal tubules and of the lower urinary tract became also more greenish (pH 6·0–6·5). The staining of the epithelium of the distal tubules slowly faded, but the colour of the collecting tubules did not change greatly. When only small amounts of sodium carbonate and bicarbonate solutions (1–2 ml. one per cent.) were injected, the colour change was less complete and was entirely reversible; but the reverse change was again so gradual that it could not be decided whether the epithelium or the lumen changed colour first. When larger amounts or more concentrated solutions of alkali were injected the kidney function was damaged.

Three frogs were prepared by previous administration of sodium carbonate and sodium bicarbonate. When the kidney was examined no marked differences could be observed in the lyochrome fluorescence in comparison with that of untreated frogs. The number of active glomeruli, however, seemed to be slightly increased. When fluorescein was injected it was traced in the renal arterioles and the glomerular capsules after about the same time interval and with the same tint of fluorescence (pH 7·5–7·0) as in untreated frogs. There was also no marked difference in the time elapsing before fluorescence appeared in the lumen of the lower parts of the urinary tract. But the green tint of the fluorescence in the proximal tubules was almost the same as in the glomerular capsule (pH 7·5–7·0). The tint of the fluorescence of the epithelium had the same or a slightly more yellowish tint than that in the lumen. There was little or no change of the green fluorescence during the passage of the urine through the proximal tubules; but the colour became more yellow (pH 6·5–6·0) in the distal third of the distal tubules, and remained so in the collecting tubules and the ureter. The epithelium of the distal tubules did not take up any stain; that of the collecting tubules fluoresced in a greenish yellow tint (pH 6·0). The dye seemed to be less concentrated in the lumen of the distal tubules, but to be eliminated rather more quickly than in normal frogs.

Experiments on Rats.

The results of experiments on seven untreated rats confirmed those previously described by Ellinger [1934 *b*, 1940].

not more acid than pH 6·0. The urine in the bladder of summer frogs in the experiments of Ellinger and Hirt [1929 *b*], Ellinger [1934 *a*, 1940], and in those now reported, was far more acid [between pH 5·5 and 4·5]. The results of experiments carried out by Montgomery and Pierce [1937] on artificially perfused surviving kidneys of frogs cannot be accepted as convincing, since Ellinger and Hirt [1929 *b*] have shown that the artificially perfused frog's kidney differs considerably from that of a living frog with regard to its staining with fluorescein and the pH of its different portions.

In winter frogs in Germany [Ellinger and Hirt, 1930 *a*] and in frogs treated with alkali the urine in the lower tubules was far less acid (pH 6·5–6·0), and under these circumstances no acidification occurred in the proximal tubules, a result in accordance with those reported by Montgomery and Pierce and by Richards. It might therefore be assumed that the findings of these investigators are due to the fact that the experiments were carried out by chance with animals whose urine for some reason did not become strongly acidified. This conclusion seems the most probable, since the very carefully performed experiments of McMaster and Elman [1928] on rats' kidneys, with litmus as indicator, suggest that the acidification occurred only in the distal tubules if the ultimate acidification of the urine was slight, but occurred in the proximal as well as in the distal tubules if the urine of the bladder was intensely acidified.

Montgomery and Pierce [1937] quote a paper by Bensley and Steen [1928] in order to show that these authors' findings were not in agreement with results of Ellinger and Hirt [1930 *a*] that acid is excreted through the epithelium of the proximal tubules of winter frogs if the glomerular function is abolished by ligature of the renal arteries. The method used by Bensley and Steen is open to criticism. Moreover, their results that phenol-red is discharged as a pale-red solution into the convoluted tubules, after arterial supply has been stopped, and is carried along to the distal tubules, where it is much concentrated and where the colour is deepened, are in contradiction with those of Montgomery and Pierce, who observed acidification and not alkalinisation in the distal tubules. Administration of alkali to English frogs produced a condition similar to that usually found in winter frogs in Germany [Ellinger and Hirt, 1930 *a*]. The only noteworthy difference is that in the latter the epithelial cells of the proximal tubules are more acid than in the former.

It may be noted that the results obtained in the present experiments with *Rana temporaria* are in agreement with those previously obtained in this country on Hungarian *Rana esculenta*.

The urine of rats kept on a mixed diet is less acid than that of normal frogs. The acidification occurs predominantly in the distal tubules. The content of the lumen of the proximal tubules is at most

tubules (fig. 3), and into a more greenish yellow in the lumen of the lower urinary tract. In rats kept on a mixed diet the results are similar, but the colour of the fluorescence in the tubular lumen is generally more green than in normal summer frogs (figs. 4 and 5). In rats to which ammonium chloride has been administered the fluorescence approximates more closely to that in normal frogs (fig. 6).

In vitro, the colour tint of a fluorescein solution depends entirely on the pH of the solvent. No other cause is known for a change of the tint of the fluorescence. Organic impurities and neutral electrolytes impair the intensity of the fluorescence, but do not alter the wave-length of the emitted light. Further, in very dilute solutions the intensity of the fluorescence bears a definite relation to the concentration of fluorescein. We may therefore conclude that the tint of fluorescence observed in living tissues indicates the pH of the tissue fluids, particularly since the colour changes observed *in vivo* after preliminary administration of acid and alkali to the animals correspond with those observed *in vitro*; the intensity of the fluorescence points to the concentration of the dye present in the various parts of the microscopic object.

The colour photomicrographs thus give evidence that in summer frogs a preliminary urine is eliminated in the glomerular capsule of the same pH as that of the blood plasma. The preliminary urine is acidified to a certain degree in the proximal tubules. Both findings are in complete agreement with the results of Ellinger and Hirt [1929 *b*] on German *Rana esculenta* in summer, and with those of Ellinger [1934 *a*, 1940] on Hungarian *Rana esculenta* at all seasons in England; the second, relating to the proximal tubules, appears to be entirely opposed to the results of Montgomery and Pierce [1935, 1937] and of Richards [1938]. This contradiction may, however, be only apparent. Montgomery and Pierce, and Richards made no experiments with the method or with the species of amphibians used by Ellinger and Hirt, while the two species used by the American authors were not available in this country nor was it possible to use their method of checking the pH in *Necturus*. Fluorescein was chosen in preference to phenol-red as indicator on account of its wide range and of its visibility in very low concentrations.

Phenol-red, as used by Montgomery and Pierce [1935, 1937] in their experiments on *Rana pipiens*, does not allow the estimation of acidities greater than pH 6·8. These authors were thus unable to supply data for the actual pH of the bladder urine in those experiments in which the acidification of urine in the tubules was examined. Findings of pH between 7·0 and 4·6 for the bladder urine of these animals are mentioned, but presumably refer to other experiments. In their experiments on *Necturus maculosus* direct measurements of the pH were made by other methods in the lower urinary tract, and it is evident that in all but one experiment reported the urine in the bladder was

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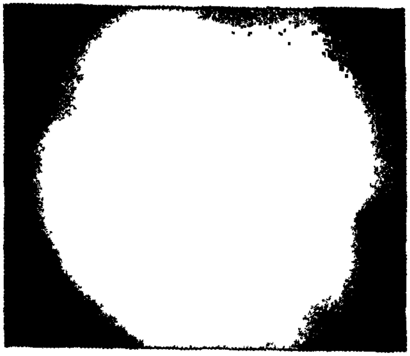

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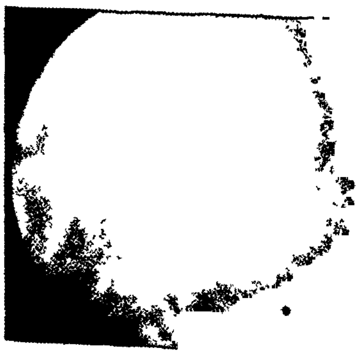

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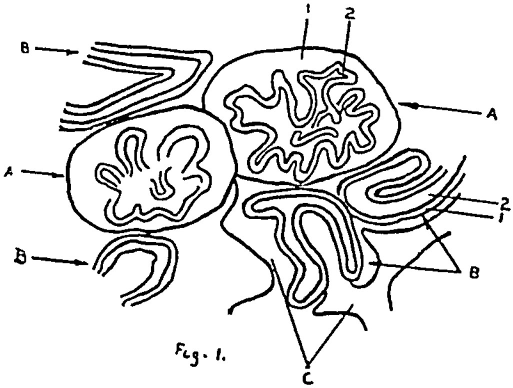
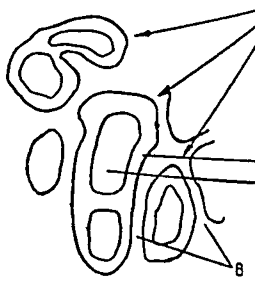


Fig. 1.



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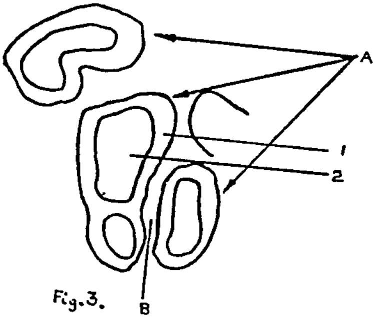

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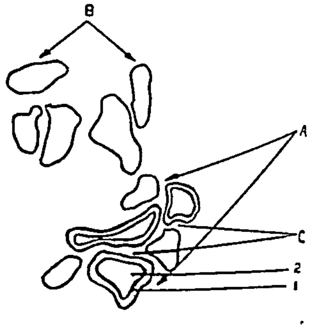

Fig 4

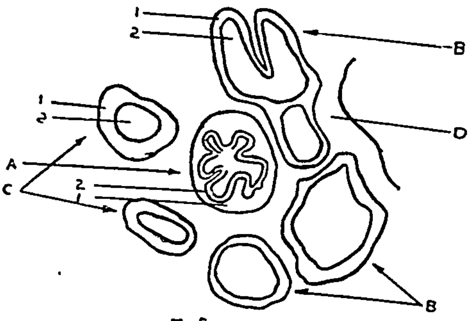

Fig 5

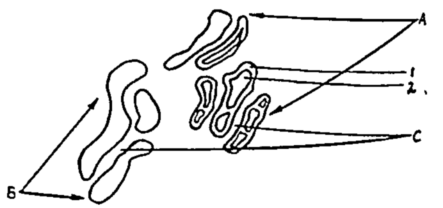

Fig 6

Key to Plate I.]

EXPLANATION OF PLATE I.

Figs. 1–6.—Colour photomicrographs of living kidneys. Magnification: figs. 1–4 and 6, ×208: fig. 5, ×400. Eyepiece: photo 4×(Zeiss). Objective: figs. 1–4 and 6, W.I. 39, num. Ap. 1·10; fig. 5, E.-H. 75, num. Ap. 1·15; both special lenses for intravital microscopy (Zeiss). Camera: Leica (reducing the magnification to 1/3). The photographs have been afterwards enlarged 4 times. Filters of the primary light source: Copper sulphate solution 10 per cent. +U.G.1, 1 mm. (Schott). Trap filters: G.G.11, 2 mm. (Schott). Film: Kodachrome X.

Fig 1.—*Rana temporaria*, ♂, kidney, ventral side, 20 min. after injection into a lymph sac of 0·5 mg. fluorescein in 0·5 ml. Ringer's solution. Active glomeruli (A) with green capsule (A1) (pH 7·2) and dark capillaries (A 2). Some distal tubules derived from active glomeruli (B) with narrow yellow epithelium (B 1) (pH 6·2) and narrow very bright yellow lumen (B 2) (pH 5·5). Some dark green veinal capillaries (C). Exp., 90 secs.

Fig. 2.—*Rana temporaria*, ♂, kidney, dorsal side, 22 min. after injection into a lymph sac of 0·5 mg. fluorescein in 0·5 ml. Ringer's solution. Proximal tubules (A) with broad green-yellow epithelium (A 1) (pH 6·5) and very broad yellow lumen (A 2) (pH 6·0). Between the tubules darker green veinal capillaries (B). Exp., 2 min.

Fig. 3.—*Rana temporaria*, ♂, same frog as in fig. 2, dorsal side, same region as in fig. 2, 1 hr. 5 min. after photograph 2 was taken and 1 hr. after the intrastomachal administration of 5 ml. of a one per cent. sodium carbonate solution and the simultaneous injection of 5 ml. of a one per cent. sodium bicarbonate solution into a leg lymph sac. Proximal tubules (A) with broad green epithelium (A 1) (pH 7·2) and very broad green to yellow-green lumen (A 2) (pH 7·0). Between the tubules darker green veinal capillaries (B). Exp., 2 min.

Fig. 4.—Rat, kidney, surface, 20 min. after intraperitoneal injection of 1 mg. fluorescein in 1 ml. Ringer's solution. Proximal tubules (A) green to yellow-green epithelium (A 1) and lumen (A 2) (pH 7·0). The tubules derived from inactive glomeruli are dark (B). Between the tubules dark green blood capillaries (C). Exp., 5 min.

Fig. 5.—Rat, kidney, after shaving off the surface, 30 min. after intraperitoneal injection of 1 mg. fluorescein in 1 ml. Ringer's solution. Glomerulus (A) with bright green capsule (A 1) (pH 7·2) and dark capillaries (A 2), distal tubules (B) with greenish yellow epithelium (B 1) (pH 6·5) and yellow lumen (B 2) (pH 6·0). Some proximal tubules (C) with green epithelium (C 1) and lumen (C 2) (pH 7·1) and dark green intertubular capillaries (D). Exp., 3 min.

Fig. 6.—Rat, treated daily for 3 days with 1 g. ammonium chloride administered in little water with a stomach-tube. Kidney, surface 25 min. after intraperitoneal injection of 1 mg. fluorescein in 1 ml. Ringer's solution. Proximal tubules (A) with greenish-yellow epithelium (A 1) (pH 6·5) and lumen (A 2) (pH 6·2). The tubules derived from inactive glomeruli are dark (B). Dark green intertubular capillaries (C). Exp., 4 min.

that this process might be involved in the mechanism of acidification of the urine.

SUMMARY.

1. A method has been described by which objective records can be obtained of the pH present in cells and body fluids of living organs by taking colour photomicrographs of the fluorescence of fluorescein injected into the animals.

2. By means of this method it has been demonstrated that in the kidneys of frogs and rats the urine eliminated into the glomerular capsules is of the same acidity as the blood plasma. This preliminary urine is acidified exclusively in the distal tubules if the ultimate acidification is moderate, but in both proximal and distal tubules if the acidification of the bladder urine reaches a higher degree

3. The mechanism of this acidification is discussed, and it is suggested that the acidification is caused probably by absorption of alkali in both sets of convoluted tubules from the content of the lumen.

I wish to express my thanks to the Lister Institute for hospitality and for a grant enabling the reproduction of the colour photomicrographs, to the Society for the Protection of Science and Learning for a Research Fellowship, and to the Ella Sachs Plotz Foundation for a grant for technical assistance. I am very much indebted to Professor R. Robison for his assistance in the preparation of the manuscript.

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only slightly more acid than that of the capsules. Administration of ammonium chloride leads to formation of a far more acid bladder urine, and in these animals acidification occurred in both proximal and distal tubules.

The site of acidification of urine is, therefore, the same in both frogs and rats. It occurs exclusively in the distal tubules if the ultimate acidification of the urine is slight, but in the proximal tubules also if the bladder urine is more acid.

It may finally be considered to what extent do these experiments throw light on the mechanism of acidification of the urine. Ellinger and Hirt [1929] assumed from the order in which injected fluorescein appeared in the lumen and epithelium of the proximal and distal tubules that the acidification was due to the reabsorption of alkaline components in the tubules. But no direct evidence was furnished for this assumption. If the body is suddenly overloaded with acid by the injection of large amounts of acid salts, either intravenously or into the lymph sac, an acid urine is excreted very rapidly through the epithelium of the proximal tubules [Ellinger and Hirt, 1931; Ellinger, 1934 *a*, 1940]. This rapid excretion can be followed directly. This excretion, however, cannot be the mechanism in use for the normal moderate acidification of urine in both convoluted tubules, since its repeated call into action is harmful for the epithelium involved [Ellinger, 1940]. *The results observed after administration of alkali do not furnish any information with regard to this question.*

Another fact makes it probable that the acidification of the urine in normal frogs and rats, in both convoluted tubules, is due to the reabsorption of alkaline components. In polyuria the urine has frequently been found more alkaline than in oliguria or under normal conditions. For example, this was found to be the case in rabbits fed exclusively with sugar solutions by McMaster and Elman [1928], and particularly by Rohde and Ellinger [1913] and Ellinger and Rohde [1921], who showed in dogs and rabbits that the urine eliminated by a denervated kidney was more plentiful and more alkaline than that of the intact kidney of the same animal. This phenomenon can only be explained by the diminished reabsorption of alkali in consequence of the accelerated passage of the glomerular urine through the convoluted tubules. *An excretion of alkali in the convoluted tubules* cannot be the cause, since, as Ellinger and Hirt [1931] have shown, the epithelial membranes of the convoluted tubules are impermeable to alkali in the direction from blood to lumen.

The present experiments threw no light upon the rôle which the renal formation of ammonia plays in the regulation of the urinary acid. The formation of ammonia in the kidney was first observed by Nash and Benedict [1921, 1922], and was found by Ellinger and Hirt [1925] to be governed by sympathetic nerves, a fact which suggests

THE INFLUENCE OF CORAMINE ON CIRCULATORY CAROTID SINUS REFLEXES.[1] By REINHARD MÜLLER. From the Ciba Research Laboratories.

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CAROTID sinus reflexes are utilised to determine the extent to which nervous control of circulatory function is involved in circulatory changes. This work was especially promoted by Heymans and Koch and their co-workers. Palme [1936] has constructed special diagrams to evaluate the effect of central stimulating drugs on the reflexes which come into operation as a result of a rise of carotid sinus pressure. The influence of Coramine on the increase in blood-pressure produced by closure of both common carotids has been investigated for the first time recently by Kayser [1938]. Cats used for the experiments were poisoned with carbon monoxide. Instead of rising in the normal manner, the blood-pressure fell after clamping off the two carotids in these animals. If Coramine was given the reflex returned to normal and there was a rise in blood-pressure, *i.e.* the reversal of the reflex which had been produced by carbon monoxide was counteracted by Coramine. In interpreting the results, Kayser [1938] expresses the opinion that Coramine, in addition to its specific action on the autonomic centres, has a vasodilator action on the cerebral blood-vessels and hence compensates the cerebral anoxæmia which has been intensified by clamping off the carotids. The anoxæmia produced by carbon monoxide is assumed to have irritated the vagal centre and hence to have caused the reversal of the reflex.

Our experiments were concerned with the action of Coramine in the narcotised animal both on the blood-pressure-raising carotid sinus reflex, which originates through clamping off the two common carotids, and on the blood-pressure-decreasing carotid sinus reflex, which is brought about by artificially increasing the blood-pressure in the carotid sinus isolated from the circulation.

The experiments described below were carried out on 66 rabbits and 10 cats. Urethane was used as narcotic. We would mention that the carotid sinus reflex varies in strength to an extraordinary degree in cat experiments. Furthermore, we succeeded only with difficulty

[1] The results given here in detail were communicated briefly to the Congress of Physiology in Zürich, 1938.

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those described in the table. All our experiments took a similar course so that a further series was not deemed necessary. The table and fig. 1 show that the reflex fall in blood-pressure due to a rise of sinus pressure is diminished after the injection of Coramine during the increase of the blood-pressure, but more or less enhanced during the period when the elevated blood-pressure returns to its normal level.

TABLE I.—CAROTID SINUS PRESSURE REFLEX IN RABBITS BEFORE AND AFTER 60 MG./KG. CORAMINE.

Reflex produced by raising pressure in one Carotid Sinus to 250 mmHg.

Curve No.	Fall of blood-pressure in mmHg before the injection (average of 3–10 experiments).	Fall of blood-pressure in mmHg after the injection.											
		1′.	2′.	3′.	4′.	5′.	7′.	10′.	12′.	15′.	20′.	25′.	30′.
309	34·5	3	3	11	22	24	37	57	42	41	35	25	22
312	31·0	11	17	24	34	34	80	88	81	84	72	59	46
315	56·0	31	29	41	60	62	77	71	77	72	61	49	50
317	25·5	38	78	72	82	89	123	51	18	45	70	52	50
327	34·0	6	9	9	9	8	11	17	12	13	6	5	3
336	26·5	9	24	27	20	23	18	50	20	25	37	24	48
337	10·5	6	6	3	4	5	19	45	39	41	56	51	50
342	35·5	35	20	35	45	48	54	53	28	48	46	39	52

The carotid sinus pressure reflex is characterised by a fall of blood-pressure from its main level to the so-called residual blood-pressure as a result of an artificial increase of pressure in the carotid sinus. Hering [1927] attaches particular importance to this residual blood-pressure. A marked increase in this residual level was observed after Coramine, and this is the reason that only a quite inconsiderable decrease of the increased main level can be obtained within the first 5 minutes by the reflex. Fig. 1 shows parts of an original curve.

During the time that the blood-pressure begins to fall to its normal level there occurs an intensified action of the reflex which can be interpreted as a predominance of the physiological regulation over the action of the medicament. In single cases there can exist an increased reflex from the beginning of the experiment. The reason for this finding is perhaps that in these animals the blood-pressure is raised by Coramine considerably more than in animals with intact presso-receptor nerves. The blood-pressure is therefore excessively increased and the fall in blood-pressure produced by the reflex becomes correspondingly greater. The restraining action of Coramine on the

in narcotising the cats anything like uniformly with the same doses of urethane. In dogs, the aortic nerve runs in the so-called vago-sympathetic, and this nerve is therefore generally severed on both sides by other authors in their experiments. The elimination of the aortic nerves is necessary for the majority of experiments according to Hering, Koch, and Heymans. We did not, however, undertake such a procedure as it would certainly have cut out a large part of the autonomic nervous system which supplies the thorax and its organs.

Carotid Sinus Pressure Reflex with Coramine.

Method.—The preparation of the carotid sinus was carried out by the method described by Koch. The aortic nerves were severed in order to maintain exclusively the reaction of the carotid sinus. For anatomical reasons mentioned above this is most safely carried out on the rabbit. The animals were narcotised with 1·0 g./kg. urethane subcutaneously. In all experiments reflex action was produced by suddenly raising the carotid sinus pressure to 250 mmHg, at which level it was maintained for 15 seconds. The choice of these experimental conditions offers, in the opinion of authoritative workers, the best means of studying the physiology of the carotid sinus [Koch, 1931]. As optical methods were used for the registration of the results, we have always been able to register a number of factors on the same animal [Meier and Müller, 1938 *a*]. The arterial pressure in the carotid artery opposite the prepared carotid sinus was registered, as well as the venous pressure in the external jugular vein and the vena cava. In the latter the registration cannula was inserted through the external iliac vein into the lower part of the inferior vena cava. Trendelenburg's [1924] counter-current apparatus and 50–60 mg. of Heparin were used per animal to prevent coagulation of blood in the cannulæ. As fluid for the Trendelenburg apparatus we used 0·9 p.c. NaCl instead of N/6 Na_2CO_3 solution, as the alkali often has a disturbing influence. All pressures both of liquid and gases were registered by means of glass plate manometers. In the majority of cases other factors were registered, *i.e.* the respiratory volume, the movement of the diaphragm, and the pressures in the body cavities. This was done with a view to obtaining as far as possible a complete record of the respiratory and circulatory responses. As this question will not be further discussed in this communication, the arrangement is not described in detail; it is analogous generally to the one described in an earlier paper [Meier and Müller, 1938 *b*].

Experimental Results.—The results of the experiments, as far as they concern arterial blood-pressure, are summarised in Table I. The table gives the figures obtained from eight experiments. A further preliminary experiment was for various reasons not so complete as

presso-receptor nerves, and it may be mentioned that a stabilisation of the blood-pressure is in general effected by Coramine.

We see, therefore, that in the animal deprived of carotid sinus and

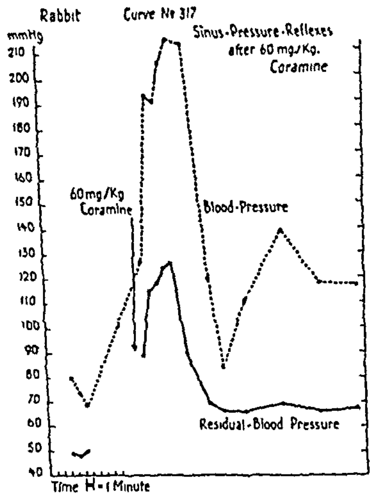

FIG. 2.—Graphic summary of experiment No. 317, rabbits, sinus pressure reflex. Marked increase of the initial pressure after Coramine. Increased reflex action, nevertheless marked and prolonged increase in the residual blood-pressure.

The circles on the curves indicate points at which the reflex was elicited.

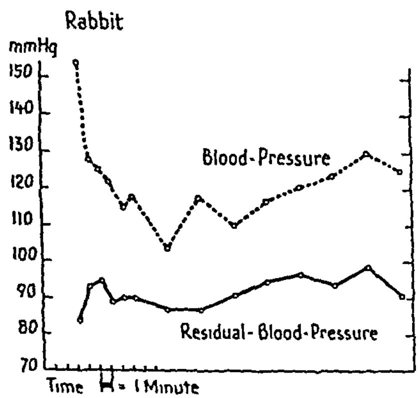

FIG. 3.—Graphic summary of an experiment without using Coramine; sinus pressure reflexes induced repeatedly during half-hour. Release of a reflex at the beginning of every minute. Later at the beginning of every third minute.

aortic nerves, the significance of the Coramine action is to be found less in the fact that it increases the blood-pressure to a greater extent than in the normal animal, than in the fact that the residual blood-pressure is quite considerably increased during the carotid sinus pressure reflex compared with normal.

carotid sinus reflex can, nevertheless, be recognised also in these cases through the fact that the reflex does not produce a fall of the blood-pressure to the normal residual level. The residual blood-pressure, therefore, is also distinctly elevated by the action of Coramine in these experiments. The importance of the phenomena described lies not only in the absolute value of the blood-pressure decrease produced by the reflex, but in the fact that the residual blood-pressure remains at a

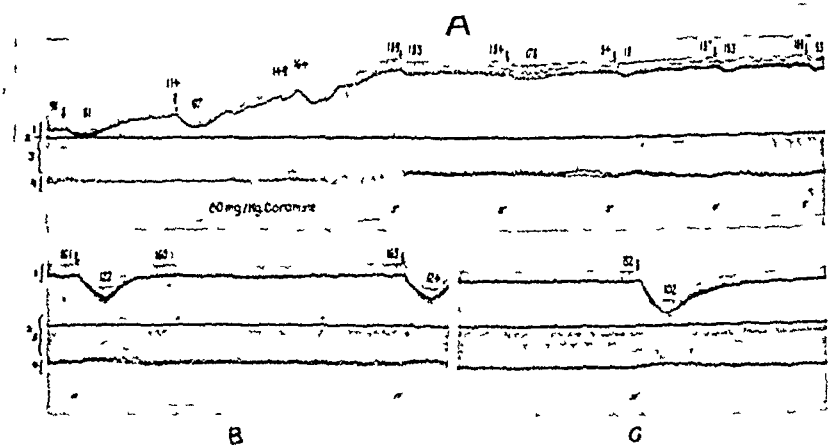

FIG. 1.—Sections of the original curve of experiment No. 337, rabbit Sinus pressure reflex.

Section A: Reflex action on narcotised animal tested at each arrow, increase of blood-pressure after Coramine and almost complete suppression of the reflex, high residual blood-pressure.

Section B: Increased reflex during the phase of the falling blood-pressure, initial and residual blood-pressure in comparison to those of the normal considerably increased, 12th, 15th, and 30th minutes.

1. Arterial blood-pressure.
2. Line of measurement.
3. Pressure in the jugular vein.
4. Pressure in the vena cava inferior.

The figures above the carotid artery-pressure denote pressure in mmHg.

higher level during the whole duration of the experiment. This is most clearly seen from the experiment reproduced in fig. 2, in which the original blood-pressure had, after a strong increase, become fairly low by the 12th minute after injection. The curve of the residual blood-pressure nevertheless runs uniformly at a higher level than normal.

As proof that these alterations produced by Coramine do not occur spontaneously, the reflex was elicited in an animal for 30 minutes and the preparation remained unaltered, as shown in fig. 3. It is a remarkable fact that the main blood-pressure shows variations in the same way as the residual blood-pressure. However, such irregularities are to be expected in an animal which has been deprived of the principal

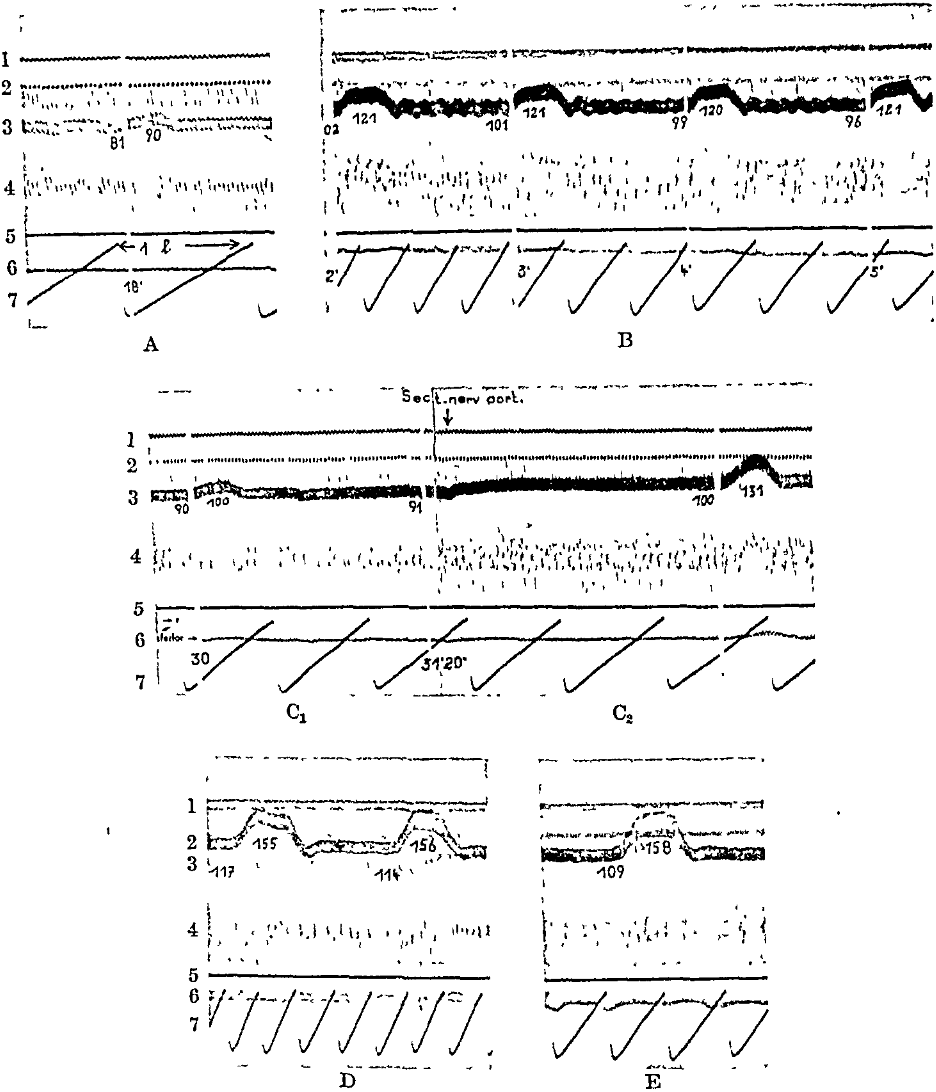

FIG. 4.—Sections of the original curves of experiment No. 480, rabbit, carotid occlusion reflex.

Section A: Normal occlusion reflex of the narcotised rabbit.

Section B: Occlusion reflex within the 2nd and 5th minutes after injection of 60 mg./kg. Coramine, with retention of the aortic nerves.

Section C_1: Normal reflex after diminution of the effect of Coramine.

Section C_2: Increase of blood-pressure after severance of the aortic nerve. Carotid sinus reflex in this condition.

Sections D, E: Occlusion reflex in the 4th, 5th, and 25th minutes after Coramine. The figures given in the blood-pressure curve show the readings of the initial blood-pressure and the reflex action. The difference between both figures corresponds in mmHg to the increase in pressure during the occlusion.

1. Intratracheal pressure.
2. Intrapleural pressure.
3. Arterial pressure.
4. Pressure in the vena jugularis.
5. Base-line.
6. Pressure in the vena cava inferior.
7. Respiratory volume (the distance between each summit = 1 litre of inspired air).

Carotid Occlusion Reflex under Coramine.

The classical and simplest examination of the normal and pathological function of the carotid sinus is made by clamping off both carotids. The resultant increase in blood-pressure is a regular phenomenon which shows only individual quantitative differences. We have carried out experiments on 55 animals (rabbits and cats) in which this occlusion reflex was examined under the effect of Coramine and under changing conditions.

Method.—In a large number of experiments the aortic nerves were severed and one of the carotid sinuses functionally eliminated through measuring the blood-pressure in this carotid, while the other was left undisturbed in its natural relation to the circulation and clamped off for periods of 15 seconds. The remarks made above, under the method given for the elimination of the carotid sinus pressure reflex, apply to the registration of other factors besides the blood-pressure.

Experimental Results.—The principal result was found to be that the blood-pressure rises after the injection of Coramine more than in the normal state on clamping off the carotid. The reflex is therefore increased by Coramine.

In the study of the carotid sinus, Koch [1931] attaches great importance to the percentage increase or decrease in blood-pressure. The value of this quantitative method was later somewhat restricted by his co-worker Palme [1936], who showed that it did not offer for pharmacological purposes the expected possibility of comparison. We have nevertheless calculated this value from a large number of experiments. The increase of the reflex due to Coramine is also seen clearly when using this type of evaluation. No increase in the occlusion reflex was observed under normal conditions over a period of one hour, so that there is no possibility of a spontaneous change, and the effects we observed may with certainty be attributed to the action of Coramine alone.

For the results described it is of no importance if the aortic nerves are retained or if they are cut. There is an increase in blood pressure which may decrease to a more or less marked extent. Fig. 4 again shows sections of an original curve.

During the normal period the blood-pressure increases, if the aortic nerve has not been interfered with, to a maximum of 14 mmHg. Coramine increases the reflex considerably, to at least 21 mmHg; 30 minutes after the injection of Coramine the main effect has subsided. If the aortic nerves are simultaneously severed, there is an intensification of the reflex, as would be expected. If Coramine is again given, the reflex is much greater than after the first dose of Coramine. The initial blood-pressure now sinks gradually, but the reflex remains

carried out in connection with another problem, showed in principle the same results. The results on cats did not show the same regularity as on the rabbit, although fig. 6 shows that similar results are obtained. Akahoshi [1938] also obtained equivocal results in his experiments on the influence of the carotid sinus on the respiratory action of Coramine.

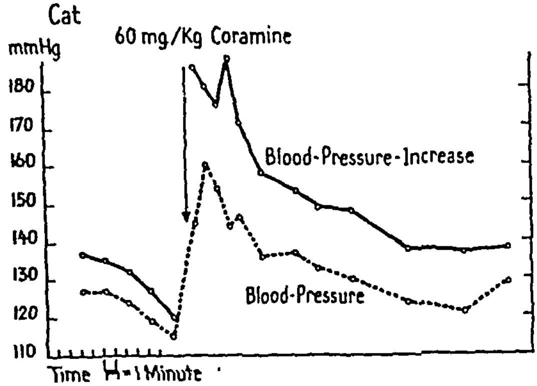

Fig. 6.—Graphic summary of the pressure obtained in an experiment carried out on the cat with the occlusion reflex and after administration of Coramine (representation as in fig. 6).

The Effect of Coramine on the Carotid Sinus.

After it had been shown that the carotid sinus is also concerned in the analeptic action on the respiration [Zunz and Tremonti, 1931], it remained to find out by experiment if Coramine has any direct effect on the carotid sinus. For this purpose one of the carotid sinuses in the rabbit was denervated, the aortic nerves severed, and the other carotid sinus arranged as a cul-de-sac. Preliminary experiments showed that only strictly isotonic solutions should be used for filling the carotid sinus. If the carotid sinus was subjected to pressure, unchanged decrease in pressure was always maintained with pharmacologically inactive solutions (NaCl, glucose). If the carotid sinus was filled with $1\frac{1}{2}$ p.c. solution of Coramine, the reflex was at first weakened and finally suppressed (fig. 7). On refilling with normal salt solution, the reflex returned completely. Weaker Coramine solutions are ineffective. The concentrations necessary for local influence in the carotid sinus are therefore much higher than the concentrations occurring in the blood of the animal, in which a pronounced blood-pressure-raising effect may be obtained by intravenous injection.

It should also be mentioned that none of the principal pressoreceptors can be directly influenced by Coramine in the type of experiments which we have used for studying the carotid sinus pressure reflex, as the aortic nerves are severed and one carotid sinus denervated. The remaining carotid sinus is eliminated from the circulation so that

increased by Coramine for more than 30 minutes and is maintained approximately at the same pressure level.

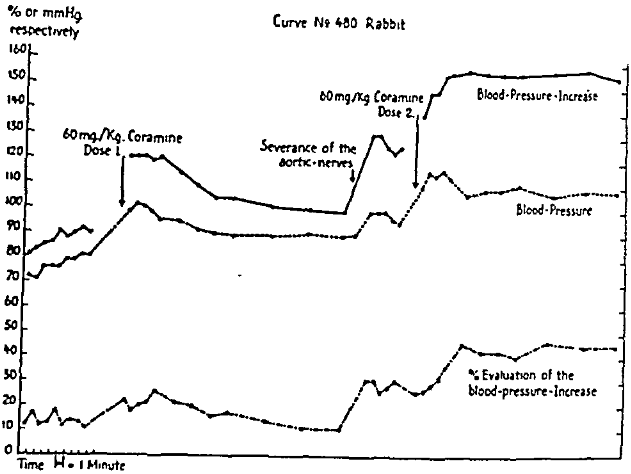

FIG. 5.—Graphic summary of all the readings of the blood-pressure and of the effects of the carotid occlusion obtained during experiment No. 480 (see original curve, fig. 4).

TABLE II.—CAROTID OCCLUSION REFLEX BEFORE AND AFTER 60 MG./KG. CORAMINE.

Curve No.	Increase of blood-pressure in mmHg before the injection (average of 3–10 experiments).	Increase of blood-pressure in mmHg after the injection.											
		1′.	2′.	3′.	4′.	5′.	7′.	10′.	12′.	15′.	20′.	25′.	30′.
292	18	..	35	29	24	29	12	18	18	14	21	17	12
295	7·5	11	5	22	2	0	14	16	15	16	16	13	14
321	16	..	42	27	32	30	38	41	41	33	30	19	15
330	26	45	37	27	29	34	37	36	51	42	..	35	48
331	11	17	17	18	22	26	33	39	35	54	56	31	20
332	4·5	31	38	27	33	40	38	37	17	33	28	8	..
333	5·5	50	39	38	51	42	36	26	24	24	..	..	..
334	6·5	70	37	37	40	50	34	19	26	6	6	..	..
335	11·5	57	29	33	22	29	31	16	23	8	11	7	..
475	6·4	10	18	18	21	9	18	14	12	12	10	9	..
479	17	27	19	13	19	23	22	21	18	18	19	17	17
480	10·5	22	19	20	21	25	20	18	14	15	12	10	10

Table II shows the values of blood-pressure changes produced by reflex action for 12 experiments. Further experiments, which were

local action of Coramine on the carotid sinus. The afferent part of the reflex arc cannot, therefore, be considered as the point of attack of Coramine.

The doses of Coramine used (60 mg./kg.) are about 1/15th–1/20th of the lethal dose for the urethanised rabbit and cannot be considered as high doses, although an analeptic action on the rabbit can be produced with about 1/100th of the lethal dose. We have chosen a dosage of 60 mg./kg. however, in order to obtain a pronounced Coramine effect. Kayser [1938] obtained his results with smaller doses (about 37 mg./kg.).

A confirmation of our results has recently been published by Burn [1939]. Unfortunately the information in his lecture is not sufficiently exact for the dosage used to be inferred. If animals weighing 3 kg. were used, then our dosage is approximately the same as that used at Burn's Institute. We cannot, however, agree unconditionally with the comparison between the dose given to animals and the largest therapeutic dose. A number of cases are described in medical literature in which a single dose of 10–20 c.c. on narcotised patients of Coramine injected intravenously produced the desired effect. For this reason 2·5–5 g. instead of 0·5 g. should be mentioned in the table as the maximal therapeutic dose. These high doses administered in cases of poisoning correspond in man to 0·05–0·06 g./kg., *i.e.* approximately the doses used by us in animal experiments.

Summary.

1. The blood-pressure-decreasing carotid sinus pressure reflex is weakened in urethanised rabbits under the influence of Coramine. An essential increase in the so-called residual blood-pressure is to be regarded as a sign of the increase in tone of the peripheral circulation.

2. Both in the urethanised rabbit and in the urethanised cat the blood-pressure-raising carotid occlusion reflex shows a larger rise in blood-pressure under the effect of Coramine than before its administration. This is also to be regarded as a sign of the peripheral vascular tonic effect. This result is obtained both with intact and severed aortic nerves.

3. Coramine produces in animals whose aortic nerve has been severed a larger rise in blood-pressure than in the intact animal.

4. The effect of Coramine on the carotid sinus itself can be excluded for the results reported. Coramine is capable only in high concentration of influencing the presso-receptors locally.

Coramine can act only centrally. It is important to emphasise this in order to clarify the conclusion that the weakened sinus pressure reflex

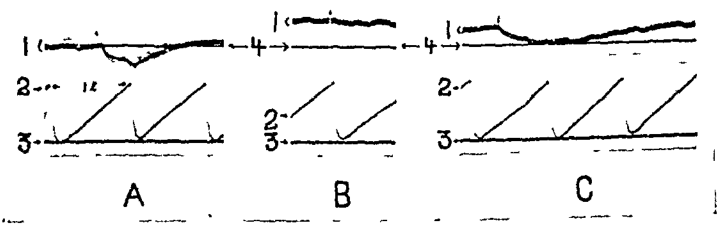

FIG. 7.—Original curve of the experiment No. 354.

A. Carotid sinus pressure reflex, normal.
B. Carotid sinus pressure reflex, after local application of a 1½ p.c. solution of Coramine.
C. Carotid sinus pressure reflex, after removal of the Coramine by washing with Ringer's solution.

1. Arterial blood-pressure.
2. Respiratory volume.
3. Intratracheal pressure.
4. Line of measurement.

cannot be explained by a direct paralysing effect of Coramine on the sensory presso-receptors.

DISCUSSION OF THE RESULTS.

Koch *et al.* [1931] are of the opinion that the effects of carotid sinus reflexes on the blood-pressure are produced by *alterations in diameter* of the peripheral blood-vessels. If this view is right, our experiments show clearly that there is, under the influence of Coramine, an actual vasoconstriction or a mild response to vasoconstrictor stimulation. In our experiments a greater increase of blood-pressure was observed than would be expected under normal conditions and was obtained with opposite kinds of stimulation, *i.e.* increase or decrease of the endosinusal pressure. The described results would indicate that Coramine has a central point of attack. The possibility can nevertheless not always be excluded that Coramine also acts peripherally on the smooth muscle of the vessels. Even if the course of the reflex is determined under normal conditions by the vasomotor centre, a fact which is probable both under pathological and pharmacological conditions, the condition of the end-organ, the peripheral circulation, is also of essential importance (*e.g.* Hess's nutrition reflex, see also Rein and Schneider, 1935). It is therefore possible that with the reflexes described, a better response could be obtained from the end-organ.

The experimental arrangement and the special tests with the direct application of Coramine to the carotid sinus exclude with certainty a

THE PERMEABILITY OF THE SYNOVIAL MEMBRANE. By D. ENGEL. From the Wilkie Surgical Research Laboratory and the Department of Pharmacology, University of Edinburgh.

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PART I.

THE significance of the synovial membrane for the blood-supply of the joints has been recognised since the time of William Hunter [1742], and it is common knowledge to-day that the articular cartilage and the joints in general are nourished chiefly by the synovial membrane and not, as thought formerly, by the blood-vessels supplying the bone. The cartilage, itself devoid of blood-vessels, represents such a dense filter that one can safely assume that practically everything that reaches the joint from the blood-stream has to pass the double membrane, consisting of the capillary endothelium and the synovial cells. I propose to call this composite membrane "barrière articulaire" in analogy to the barrière rachidienne of the choroid plexus. The bulk of the normal food-supply, foreign substances circulating in the blood, as well as drugs injected intravenously, have to pass this barrière to reach the joint cavity and the cartilage, hence its importance from the standpoint of physiology, pathology, and pharmacology.

In spite of these facts, very little is known about the functional qualities of this barrière. Resorption from joints has been repeatedly the subject of study, but the reverse process, excretion from the blood into the joints, from the practical standpoint the more important route, has been conspicuously neglected. The scanty data available in the literature are based chiefly on observations made on inflamed joints which, as will be shown later, behave differently from the normal ones. When one considers that M. W. Ropes *et al.*, in their comprehensive study of the literature in 1939 came to the conclusion that even "the physical and chemical properties of normal synovial fluid have never been well established," it will be realised that little progress has been made since Bichat [1799] made the statement: "Aucune partie de la physiologie des os n'abonde plus d'hypothèses et moin en decouvertes que l'histoire du système synovial. Beaucoup de dissertations et peu de faits."

The only systematic experiments on excretion of substances from

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PART I.

THE significance of the synovial membrane for the blood-supply of the joints has been recognised since the time of William Hunter [1742], and it is common knowledge to-day that the articular cartilage and the joints in general are nourished chiefly by the synovial membrane and not, as thought formerly, by the blood-vessels supplying the bone. The cartilage, itself devoid of blood-vessels, represents such a dense filter that one can safely assume that practically everything that reaches the joint from the blood-stream has to pass the double membrane, consisting of the capillary endothelium and the synovial cells. I propose to call this composite membrane "barrière articulaire" in analogy to the barrière rachidienne of the choroid plexus. The bulk of the normal food-supply, foreign substances circulating in the blood, as well as drugs injected intravenously, have to pass this barrière to reach the joint cavity and the cartilage, hence its importance from the standpoint of physiology, pathology, and pharmacology.

In spite of these facts, very little is known about the functional qualities of this barrière. Resorption from joints has been repeatedly the subject of study, but the reverse process, excretion from the blood into the joints, from the practical standpoint the more important route, has been conspicuously neglected. The scanty data available in the literature are based chiefly on observations made on inflamed joints which, as will be shown later, behave differently from the normal ones. When one considers that M. W. Ropes *et al.*, in their comprehensive study of the literature in 1939 came to the conclusion that even "the physical and chemical properties of normal synovial fluid have never been well established," it will be realised that little progress has been made since Bichat [1799] made the statement: "Aucune partie de la physiologie des os n'abonde plus d'hypothèses et moin en decouvertes que l'histoire du système synovial. Beaucoup de dissertations et peu de faits."

The only systematic experiments on excretion of substances from

the blood into the joint cavity are those of Shinkichi Tani [1935]. They came to my knowledge while my experiments were in progress. His results are in some respect contrary to mine; they will be discussed later. It is surprising also that a systematic experimental study of the pharmacology of joints has not been made. Little is known to what extent drugs reach the joint cavity. At the present time the whole therapy of joints is based on empiricism. The method devised in this paper should therefore be of therapeutic value.

The first series of experiments were designed to determine what substances injected into the blood-stream reach the joint cavity, or, in other words, penetrate the articular barrier.

Dyes were injected intravenously or intramuscularly in cats and rabbits; the knee joint was then perfused with Ringer's solution, and the dye in the outflowing fluid estimated quantitatively by a colorimetric method. If the quantity of the excreted dye was very poor the perfusate was concentrated on the water-bath. The knee joint was always used for perfusion, the other joints being too small or inaccessible for anatomical reasons.

Methods.

The animals were anæsthetised with Nembutal or Sod. Amytal (40–70 mg. pro Kg.) and fixed to a board with the hind legs extended. A cannula was then inserted into the joint on the medial side of the patella and the perfusion started. After the joint had been filled with 2–3 c.cm. of fluid, a similar needle was inserted on the lateral side of the patella, and the perfusate collected. The joint cavity of a medium-sized cat or rabbit holds approx. 2–4 c.c. The articular recess reaches about 1–2 cm. above the upper margin of the patella, and by palpating this edge no difficulty is encountered in getting into the joint cavity. Great care was taken to avoid any bleeding in order to keep the barrier intact. Only blood-free perfusates were considered as satisfactory. The needle should not be too coarse, to avoid trauma, but should have a bore wide enough to allow passage of the first few drops of synovial fluid. It can be judged, from the clear perfusate, from the absence of a para-articular œdema, from the first drops of synovial fluid, and from the free communication between the two needles that the needle is actually in the joint and not in the para-articular tissue. It is advisable, however, to ascertain the position of the needles at the end of the experiment.

The needle carrying the inflowing fluid was connected by rubber tubing with a bottle containing Ringer's solution. The rate of perfusion was regulated by the level of the bottle at 10–15 drops per minute. The rubber tube passed through an electrically heated jacket which maintained the fluid at body temperature. In later experi-

ments in which a constant and equal rate of perfusion was needed, a more elaborate apparatus was used, which will be described later. The perfusion was carried out for 1–4 hours. Each animal was used only once in order to ensure normal conditions. A joint once perfused has to be considered as inflamed.

In the choice of the dyes used, experience gained in former experiments performed on the peritoneal cavity [Engel and Kerekes, 1927] was available. The dyes varied in their diffusibility and in their chemical character. The diffusibility was determined by the following method: glass tubes were partly filled with 4 p.c. aqueous gelatine solution, and after solidification, 1 c.c. of equimolecular dye solution was floated on the surface. A series of dyes were tested and the diffusibility of the dye was expressed in terms of mm. of penetration in 24 hours.

Most of the animal experiments were repeated, some of them several times, according to their importance. Again it was considered important to test the effects of injecting an acid and alkaline dye in the same animal simultaneously, in order to eliminate individual variations, and to observe the excretion of these different groups of dyes under identical conditions. In such cases dyes were selected which did not interfere with each other, for instance, Fuchsin S. and Methylene blue. With this combination the Fuchsin S. appears in the perfusate in the colourless carbinol form and does not hinder the detection of the Methylene blue, should it appear. The Fuchsin S. can then be regenerated in the coloured form by adding a few drops of acetic acid to the perfusate. In these combined experiments two dyes of the same or similar diffusibility were used. It was considered futile to compare quantitatively the dye in the perfusate with that in the blood, because an error may arise due to excretion of dye during the observation by the kidneys and the liver, and this may exceed the amount in the perfusate. This error varies also according to the dye used.

Results.

Only a few experiments will be described here in detail.

Experiment 1.—At 11 a.m. 60 mg. Sod. Amytal injected intramusc. in a cat of 2·4 kg.

At 11.50 a.m. 10 c.c. of a 2 p.c. aq. *Fuchsin S.* solution injected into the muscles of the foreleg.

At 11.55 a.m. perfusion of one knee joint with Ringer's solution started.

At 12 noon perfusate shows strongly red colour after addition of a few drops of acetic acid, and remains so till 3 p.m., when the animal was killed. The 2 needles were found in a satisfactory position in the

joint cavity. The amount of the collected perfusate was 60 c.c. and its colour intensity equalled that of a standard solution of 1/20000.

Experiment 2.—The same experiment as No. 1 repeated under identical conditions, with the only difference that 15 c.c. of a 1 p.c. *Methylene blue* solution were injected intravenously. The perfusion was carried on for four and a half hours. No trace of dye could be detected even in the concentrated perfusate. The same experiment repeated several times gave always the same result. Similar experiments to the two above described were performed with a series of dyes. The results are given in Table I.

TABLE I.—RELATION BETWEEN CHEMICAL CHARACTER, DIFFUSIBILITY AND RATE OF PASSAGE THROUGH SYNOVIAL MEMBRANE OF VARIOUS DYES.

Name of dye.	Chemical character.	Penetration in 24 hours in millimetres.	Appearance in perfusate.
Fuchsin S. .	acid	24·4	+ + + +
Naphthol yellow.	,,	20	+ + + +
Echtgelb	,,	20	+ + + +
Methyl orange	,,	19·4	–
Tartrazin	,,	18·5	+ + +
Cyanol	,,	18·3	+ +
Orange G.	,,	17	+ + + +
Red-violet	,,	14	–
Ponceau	,,	13·6	+ +
Eosin	,,	12·5	+ +
Patent blue	,,	10·4	–
Light green	,,	10·1	+
Brillant-crocein	,,	5	+
Anilin blue	,,	4	+
Scharlach R.	,,	0	+
Trypan blue	,,	3·9	–
Water blue	,,	1·5	–
Methyl green	alkaline	32	–
Brillant-crocein-blue	,,	29	–
Methylene blue	,,	25	–
Fuchsin basic	,,	25	–
Janus green	,,	20	–
Uranin	,,	22	–
Chrysoidin	,,	20	–
Rhodamin G.	,,	19	–
Methyl violet	,,	16	–
Congo-rubin	,,	10	–

The dyes in Table I. fall into two groups, acid and alkaline dyes; they are given in the order of their diffusibility, and were chosen so that their diffusibility covered a wide range. The study of Table I. reveals two important facts: First, *no alkaline dye has appeared in the*

perfusate, irrespective of its diffusibility. Secondly, the great majority of *the acid dyes appeared in the perfusate; the quantity present tended to be in direct proportion to their diffusibility.* For example, the basic dyes Methyl green and Brillant-crocein-blue penetrate gelatine more rapidly than do any of the acid dyes, but they do not appear in the joint perfusate. The two dyes basic and acid Fuchsin are closely allied chemically and penetrate equally into gelatine, but whilst acid Fuchsin passes freely into the synovial fluid, basic Fuchsin does not do so. The basic dye Brillant-crocein-blue and the acid dye Brillant-crocein provide a still more striking contrast, since the former penetrates gelatine rapidly but does not enter the synovial fluid, whilst the latter penetrates gelatine very slowly and yet passes into the perfusate.

The acid dyes permeate the synovial membrane, provided that their diffusibility exceeds four millimetres, *i.e.* their capacity to penetrate into gelatine in 24 hours. For instance, the acid but colloidal Trypan blue and Water blue do not penetrate the synovial membrane, but the highly diffusible Fuchsin S. or Naphthol yellow could be detected in abundance in the perfusate as early as 5–10 minutes after being injected intravenously.

There are, however, three dyes that did not comply with the general rule, they did not appear in the perfusate in spite of being highly diffusible and acid in character; they were Patent blue, Red-violet and Methyl orange. The reason of their non-appearance will be discussed later.

In a series of double experiments in which one acid and one alkaline dye were injected into the same animal, the following combinations were given: Fuchsin S. with Methyl green; Fuchsin S. with Methylene blue; Eosin with Brillant-cresyl-blue and Brillant-crocein with Methyl violet 6 B. *In each of these experiments the acid dye appeared in the perfusate as usual, while the alkaline dye was constantly missing.* One of these experiments will be described as an example:

Experiment 3.—At 11 a.m. Sod. Amytal injected intramusc. into a cat. At 12.50 p.m. 15 c.c. 1 p.c. Methylene blue was injected into the jugular vein; a few minutes later the same amount of Fuchsin S. was injected intravenously and perfusion of the knee joint was started in the usual manner. At 1 h. the perfusate was deep red after addition of a drop of acetic acid: no trace of blue colour was to be seen. The perfusion was carried on till 5.20, with the same result as at the beginning. (Addition of H_2O_2 to regenerate possible leuco form of Methylene blue does not change the colour.)

One can thus definitely conclude that the behaviour of the acid dyes is essentially different from that of the alkaline ones.

Discussion.

The first question that arises is, what is the reason for the selective appearance of the acid dyes in the joint perfusate and the conspicuous absence of the alkaline ones under identical conditions? Is this fact due to the selective activity of the barrier, or is there any other explanation for it?

It may be mentioned here that similar experiments to those described above have been previously performed [Engel and Kerekes, 1927] in the peritoneal cavity and the subarachnoideal space [Wittgenstein and Krebs, 1926]. The results were similar to those recorded for the joints. It was found that no intravenously injected alkaline dye appeared, in contrast to the acid dyes, in the cerebrospinal fluid or the peritoneal perfusate. The quantity of the acid dyes was in direct proportion to their diffusibility. This fact, that three membranes so widely diverse in their function behave so similarly, strongly suggests that the reason for the selective discrimination between acid and alkaline dyes is not the selective activity of those three membranes. Details on this question have been discussed at length by Engel and Kerekes in connection with the peritoneal permeability. Only a few points pertinent to our present problem should be mentioned here.

The alkaline dyes are, as a rule, lipoid soluble substances and for this very reason they not only enter into the cells with ease, but they are also retained by the cells. The contrary applies to the acid dyes: these are, with a few exceptions, lipoid insoluble, and therefore they are not retained by the cells, nor are they anchored to the tissue. In other words, there is a much greater affinity between cells and an alkaline dye than between cells and an acid dye. The result is that if one injects intravenously an alkaline dye, it will disappear from the blood-stream into the tissues in a very short time. It was found [Wittgenstein and Krebs, 1926] that the acid dyes need as many hours for disappearing from the blood as the alkaline ones need minutes. As much as 99 p.c. of the latter disappear from the blood in a few minutes. This is the reason why these dyes do get the chance of being excreted by either of the three membranes mentioned above. A substance that does not reach the barrier cannot pass it, and consequently does not appear in the perfusate. On the other hand, an acid dye, not being retained by the tissues, will reach the barrier, and will therefore appear in the perfusate. The question whether it is the synovial membrane that discriminates so strictly between acid and alkaline dyes can therefore be answered in the negative. The selective activity is a function of the tissues in general.

The case is quite different with regard to the selection between highly diffusible and colloidal dyes of acid character. We have seen that not all acid dyes were excreted, but only those with a diffusibility

higher than 4 millimetres. We have seen further that the quantity excreted was approximately in direct proportion to their diffusibility. As the acid dyes are not retained by the tissues, it is reasonable to assume that the variation in the degree of their permeation is due to a quality of the synovial membrane itself.

There is one fact which deserves our special attention: We saw that Methyl orange does not appear in the perfusate in spite of the fact that it is an acid dye, and according to its diffusibility it should permeate the synovial membrane, just as it permeates, as shown earlier, the peritoneal membrane. Incidentally Methyl orange is a very exceptional dye, being one of those few which, in spite of being acid in character, is lipoid soluble. (Acid dyes are, as a rule, lipoid insoluble.) Its appearance in the peritoneal perfusate is a sure indication that this dye does not disappear from the blood circulation, as may be expected from its lipoid solubility, but follows the rule of its chemical character. The conclusion seems therefore justified that the absence of Methyl orange from the joint perfusate is due to a special quality of the articular barrier, in distinction to other membranes.

It should, however, be mentioned that this is, so far, the only finding contrary to the concept of Ropes *et al.* [1939], and supported also by my own experiments, that the synovial fluid is a dialysate of the blood plasma. The authors based their conclusions on exact studies of the distribution of electrolytes and non-electrolytes between serum and synovial fluid. They think that the effect of mucin on the colloid osmotic pressure and calcium concentration of synovial fluid indicates that mucin, in addition to its lubricating action, also plays a rôle in the exchange of water and other substances between the vascular system and the joint cavity. It is therefore a possibility that the non-appearance of Methyl orange in the perfusate is due to the presence of mucin in the synovial fluid.

It was mentioned that our knowledge on the permeability of the synovial membrane is very incomplete. A. Policard [1936], reviewing the literature up to 1936, more especially the works of Gaglio, Draganesco, Pacetta, Granel, Allison and Cajori, came to the conclusion that a revision of the results of these authors would be desirable. Most of their observations concern human joint exudates.

The only systematic experiments on the subject I have been able to discover are those of Shinkichi Tani [1935], whose results differ from mine. The discrepancies between our results cannot be explained by Tani's different technique. He injected, as I did, into rabbits a group of dyes intravenously, and punctured the knee joint $\frac{1}{2}$, 1, 2 and 3 hours afterwards, washing it out with 1 c.c. of saline. He does not mention whether his animals were immobilised or ran freely between the punctures. His results obtained with the acid dyes are similar to mine; but, contrary to my experience, he claims that 16 out of 26

alkaline dyes were excreted into the joint cavity. He admits, however, that the excretion of the alkaline dyes was poor, but he attributes this to the fact that alkaline dyes are more toxic than the acid dyes and therefore lower doses had to be injected of them. That this is not the reason is proved by my double experiments, one of which (No. 3) was fully reported. We see there that the same amount of Methylene blue and Fuchsin S. were injected into the same animal, yet only Fuchsin S. appeared in the perfusate, while Methylene blue was absent from it during the whole perfusion, lasting for 4 hours. The real reason for the different behaviour of the acid and alkaline dyes is the quick disappearance of the latter from the blood circulation in contrast to the acid ones, as explained above.

The only conceivable explanation of the fact that Tani detected alkaline dyes in the synovial fluid seems to me the following: The synovial fluid consists partly of detritus cells from the coating membrane. These cells retain and contain alkaline dyes just the same as any other tissue. They are rubbed off the surface by the movements of the limb, unless the animal is immobilised. (Tani does not mention this fact.) By liquefaction of these cells the dye is set free. This small amount of dye may be demonstrable in 1 c.c. of fluid, as reported by Tani, and may account for his different results.

The fact that only acid dyes penetrate into the joint may explain why, in diseases like gout and ochronosis (Virchow), uric and homogentisic acid respectively are found in excess.

Part II.

The Permeability of the Inflamed Synovial Membrane.

For practical and theoretical reasons the question whether the permeability of the inflamed synovial membrane is identical with that of the normal membrane is of considerable interest. It is evident that in therapy we have to deal with abnormal conditions, mostly with inflamed joints, and therefore it seemed of importance to know whether the conclusions drawn from the previous experiments can be applied unrestrictedly to inflamed joints. But it was also of interest to know how far the findings arrived at in inflamed joints can be generalised. It has been mentioned that the few data available in literature concerning drugs demonstrated in joint fluids were gained from exudates, *i.e.* inflamed joints.

Technique.

Rabbits were chiefly used in which 1–2 c.c. of a 1 p.c. Lugol solution was injected into one knee joint, the other being used as a control. One or two days after the injection the two joints were perfused simul

taneously with Ringer's solution, as described in the previous experiments. Only once was a staphylococcus aureus suspension used for producing an inflammation, the method being abandoned because it provoked an ulcerative arthritis, with blood in the perfusate, and was therefore unsuitable for testing the permeability of the barrière. The joint surface, examined two days after the Lugol injection, did not look macroscopically inflamed.

A description of one experiment suffices to illustrate the results obtained:

Experiment 4.—The morning of the 14th September 1937 injection of 2 c.c. of a 1 p.c. Lugol solution into the right knee joint of a rabbit of 2 kg. On the 16th September 1937 the right knee looked swollen and œdematous.

At 9.30 a.m. intramuscular inj. of 4 c.c. 2 p.c. Nembutal.

At 10.30 a.m. animal strapped on the back with the hind legs extended equally. Intravenous inj. of 7 c.c. *Prontosil* red (Bayer).

At 11 a.m. perfusion of the two knee joints with Ringer's solution in the usual manner. Special care was taken that the rate of perfusion on the two sides should be equal.

At 11.10 a.m. an additional 3 c.c. of Prontosil inj. subcutaneously.

At 11.30 a.m. the perfusate of the normal left side strongly pink, while that of the inflamed right side perfectly free of stain. The skin and urine are intensely red.

At 1.20 p.m. red colour diminishing on the left side; no colour on the right side.

At 2 p.m. staining on the left side only in traces, the right side free of stain.

Animal killed. The total perfusate on each side 100 c.c.

P.M.: The 4 needles were intra-articularly placed. The right joint is a little congested, no ulceration.

Result.—During the whole experiment the normal joint excreted Prontosil abundantly, while the inflamed side did not show any excretion at all.

A similar experiment was repeated with a cat, four days after the Lugol injection, with Fuchsin S. as a test dye. The result was similar to that of the former experiment: a decreased dye excretion of the inflamed side. A comparative colorimetric measurement of the two perfusates showed that the perfusate of the normal side contained twice the concentration of dye as compared with the inflamed side. In a third similar experiment the ratio was 1·5 to 1. In one experiment only, in which Staphylococcus was used for producing inflammation, was the difference between the two sides in doubt. But in this case that perfusate was blood-stained and the result therefore unreliable. In all the other 8 rabbits and cats *the inflamed side excreted considerably less dye than the normal side.*

Comment.

One may rightly ask what is the explanation for this unexpected result? The blood-flow towards an inflamed tissue, in this case the synovial membrane, is considerably increased as compared with the normal. Consequently the amount of dye carried towards the membrane should be increased too. One should expect therefore an increase in the amount of the dye excreted on the inflamed side. But the contrary is the case, as we have seen. There ought therefore to be a factor offsetting the prospective result of the hyperæmia.

Experiment 5.—To ascertain whether the blood-volume reaching the synovial membrane is actually increased or decreased on account of a stasis, the temperature of both quadriceps muscles was taken in a rabbit which had one knee joint injected with 1 c.c. 1 p.c. Lugol sol. previous to the experiment. The temperature was measured by thermo-couples inserted into the two muscles pointing towards and nearly reaching the knee recesses. The temperature was recorded every 5 minutes for two hours, while the perfusion was going on with the usual technique. Four different points of the muscles were tested. During the whole time the temperature on the injected side was higher by 2° C. At the same time the dye excretion on this side was reduced to 1/7 compared with the normal side.

This experiment shows that in spite of a bigger blood-supply of the inflamed side, indicated by its higher temperature, the dye excretion was reduced. This decrease could therefore hardly be attributed to a stasis. This conclusion is borne out also by the statement of Landis that stasis is the surest sign of increased capillary permeability. The decreased dye excretion of the inflamed knee must therefore have another cause: it is, in my opinion, the inflammatory change of the synovial membrane.

For this reason it seemed of interest to examine macro- and microscopically the synovial membranes of joints injected with Lugol solution 24, 48, and 72 hours after injection. Five knee joints were examined for this purpose, the normal side serving as a control. Macroscopically the inflamed joint was a little enlarged; the synovial membrane showed engorgement of the blood-vessels; the cartilaginous bone ends were stained yellow by the Lugol sol.; the synovial membrane was not ulcerated or covered with a fibrinous coat.

In the histological section (Hæmatoxylin-Eosin, Mallory's connective tissue stain, and Weigert's stain for fibrin), the nuclei of the synovial cells appeared faded. The subsynovial connective tissue layer was œdematous and infiltrated with white blood-cells, chiefly leucocytes. The infiltration continued between the adjacent muscular bundles which showed, in parts, hyaline degeneration (see fig. 2). By Weigert's stain fibrin could be detected in the areas of infiltration.

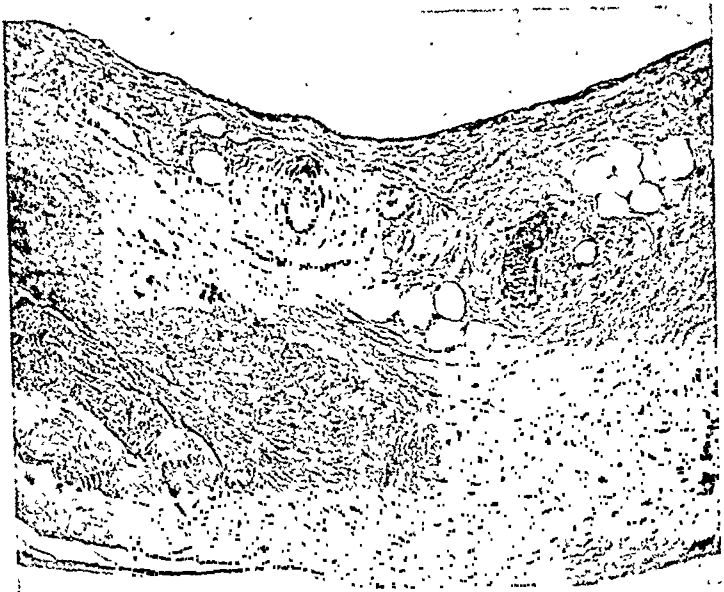

FIG. 1.—Section through normal synovial membrane and adjacent subsynovial layers. (Hæmatoxylin-Eosin. ×100.)

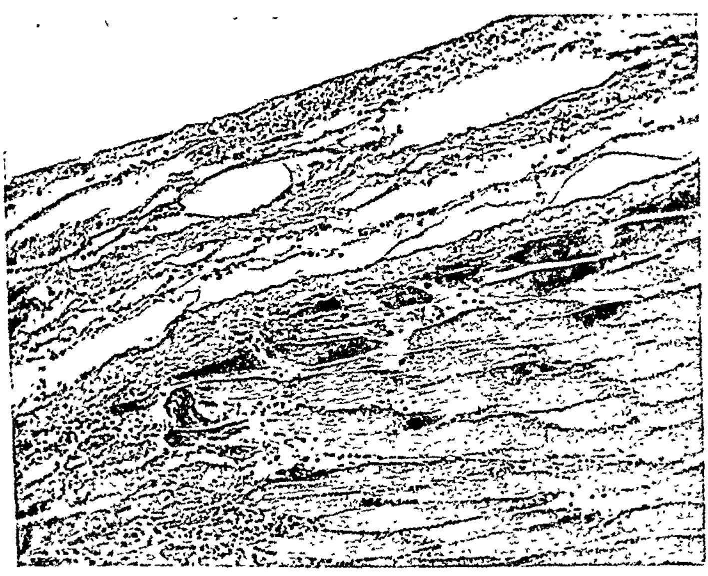

FIG. 2.—Section through synovial membrane and adjacent layers, 48 hours after intra-articular injection of 1 p.c. Lugol solution. Note the less marked staining of the synovial cells, the strong subsynovial œdema, the infiltration with white blood-cells, which extend between the hyaline degenerated muscular bundles. ×100.

All these inflammatory signs were more marked in the sections 48 and 72 hours after the Lugol injection. This histological finding supports the view that the decreased dye excretion of the inflamed joint is due to the local change in the synovial membrane.

The observation on the reduced dye excretion of inflamed joint membranes might be of some practical significance: it may not be difficult to determine and standardise the time and quantity of dye excretion of a normal joint. A delayed or reduced excretion, compared with the standard figures, would, under certain circumstances, indicate that a dubious joint is inflamed. The method would be analogous to the diagnostic functional test of the kidney (Indigo-carmin).

Keller [1914], Curtis and Brandenburg [1930], and Kling [1938] injected phenol-sulphophthalein into the knee joint, with a similar aim, and tested the excretion of the drug in the urine. They found that in chronic synovial involvement the excretion was prolonged, in acute inflammations it was accelerated.

With regard to the lack of an experimental joint pharmacology, the usefulness of the joint perfusion has been tested in respect of excretion by the synovial membrane of one or other of the most commonly used drugs in the therapy of articular diseases. A report on this test, although incomplete, seems justifiable, as the few data available in the literature on the subject refer to inflamed joints, and can therefore not be used for quantitative comparative purposes, as shown in our experiments.

One of the drugs tested was Sod. salicylate.

Experiment 6.—At 12 noon: Rabbit of 2·1 kg. Intravenous injection of 9 c.c. of a solution of 5 g. Sod. salicylate in 30 c.c. aq. dest. and intraperitoneal inj. of 5 c.c. of the same solution.

At 12.15 p.m. perfusion of the knee joint with Ringer's solution in the usual manner.

From 12.15 p.m. to 12.25 p.m. 4 c.c. of the perfusate collected (portion I.).

From 12.25 p.m. to 12.50 p.m. 15 c.c. of perfusate collected (portion II.).

From 12.50 p.m. to 2.10 p.m. 150 c.c. of perfusate collected (portion III.).

At 2.20 p.m. animal died.

The Sod. salicylate was estimated by the colorimetrical method by Dr. Spragg (Laboratory of the Royal College of Physicians, Edinburgh).

In portion I. 3·2 mg. Sod. salicylate pro 100 c.c.
,, ,, II. 0·9 mg. ,, ,, ,,
,, ,, III. only very faint traces of Sod. salicylate were found.

The experiment shows that considerable amounts of the administered drug reached the joint cavity. In ten minutes, one half-hour

after the intravenous injection of 2·5 g. Sod. salicylate, 0·28 mg. were excreted into the joint. If we consider the small surface of the joint cavity and the small amount of blood supplying this surface, in proportion to the total blood, the excreted quantity appears rather large. Another fact elucidated from the experiment is the rapidly decreasing amount of the excreted drug. While the perfusion during the first 10 minutes yielded 0·28 mg. Sod. salicylate, the second portion contained only 0·15 mg. after perfusing for 25 minutes, and the third portion only traces after further 80 minutes. The reason for this rapid decrease is probably the quick disappearance of the Sod. salicylate from the circulation.

The excretion of Sod. salicylate has been investigated previously by other authors [Stockmann, Fillippi, etc.]. Bondi and Jacoby [1924] reported that joints of rabbits infected with staphylococcus contained more salicylate than those of healthy animals. Hanzlik does not accept their evidence as conclusive. He, with Scott and Thoburn [1917], comes to the conclusion that "for all practical purposes, the alleged selectivity of salicylates for inflamed joints does not exist. If anything, the results of Froehlich and Singer and of Scott, Thoburn and Hanzlik [1917] suggest that the swollen and inflamed membranes of the synovia act as barriers to the diffusion of the salicylate." This latter interesting finding confirms our observation concerning the diminished dye excretion by inflamed joints. From other drugs of practical importance only Prontosil was tested. It appeared in the perfusate in abundance, as described in Experiment 4.

These two drugs should serve as examples that the technique used in our experiments may be useful for solving problems concerning pharmacology of joints.

Summary.

The permeability of the synovial membranes was studied by injecting intravenously a series of dyes in rabbits and cats, and perfusing the knee joints afterwards.

It was found that only dyes of acid character reach the joint cavity and appear in the perfusate. The amount excreted was in direct proportion to the diffusibility of the dye.

Alkaline dyes were not excreted into the joint cavity, irrespective of their diffusibility. The importance of this fact for joint pathology is stressed.

The permeability of the inflamed synovial membrane is considerably diminished compared with the normal. It is proposed that this fact should be utilised as a functional test of joints for diagnostic purposes, in analogy to the dye excretion tests of the kidney.

Sod. salicylate and Prontosil appeared in the perfusate in large

quantity. It is suggested that the method of joint perfusion offers possibilities for building up a systematic pharmacology of joints.

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THE RATES OF ACTION AND CLEARANCE OF BARBITURATES.

By S. C. DAS. (From the Department of Pharmacology, University of Edinburgh.)

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IT has been shown [Das and Raventós, 1939] that the rate of clearance of sodium evipan can be measured conveniently by continuous intravenous injection into mice.

The details of this method are as follows. An initial dose capable of producing 10 to 20 minutes' sleep is given intravenously. The needle is left in the vein and secured to the tail with adhesive plaster. An apparatus [described by Das and Raventós, 1939] that will provide continuous injections at a rate of about 1/50 c.c. per min. is attached to the needle and continuous intravenous infusion is carried out. The technique is very simple but it is essential to keep the mice warm since their body temperature rapidly falls to the level of the surrounding temperature.

The theory of the method has been described previously [Das and Raventós, 1939]. A constant fraction of the drug present in the body is removed per minute and hence the rate of infusion which maintains unchanged the narcosis produced by the initial dose indicates the fraction of the initial dose which is cleared per minute. In the case of sodium evipan it was found that about 1/30 of the initial dose was cleared per minute [Das and Raventós, 1939, Table VI.].

Similar experiments in the case of pentothal sodium gave the results shown in Table I. In this case it was found that the solutions deteriorated after 2 or 3 hours and that reliable results could only be obtained with perfectly fresh solutions.

The results show that the effects produced by 40 mg./kg. pentothal sodium are maintained by 0·8 to 1·0 mg./kg./min.; hence the rate of clearance is 1/40 to 1/50 per min. which is slower than the rate of clearance of sodium evipan.

Similar experiments of this type with nembutal were less satisfactory. An initial dose of 40 mg./kg., which was one and a half times the median hypnotic dose, only produced sleep after 2 or 3 minutes, a delay which lessened the accuracy of the method. Continuous infusion of 0·5 mg./kg./min. caused increasing depression and finally death, whilst recovery occurred with a continuous infusion of 0·25 mg./kg./min.

The rate of detoxication therefore lay between 1/80 and 1/60 per min. of the quantity present.

In the case of soluble barbitone it was found that the medium hypnotic dose only produced hypnosis after about 20 min. and hence the method was inapplicable.

TABLE I.—CONTINUOUS INFUSION OF PENTOTHAL INTO MICE.

Initial dose, mg./kg.	Continuous dose, mg./kg./min.	No. injected.	Fate.
30	10	3	3 deaths. Average time 18 min.
30	5	3	2 deaths. Average time 50 min. 1 depression increased.
30	1·2	3	3 depression increased.
30	1·0	2	2 depression increased.
40	1·0	3	1 depression increased. 2 depression steady.
40	0·8	3	1 depression steady. 2 awakening.

RATE OF ACTION OF BARBITURATES.

Sodium evipan and pentothal sodium, when given intravenously, produce their action very rapidly. For example, in the former case hypnosis is produced in one or two minutes by the median hypnotic dose and death in a few minutes by the median lethal dose. In the latter case, however, a certain number of delayed deaths occur from ½ to 1 hour after the administration.

In the case of nembutal, which has a more prolonged action, the rate of action is distinctly slower. This fact is shown in Table II. The median hypnotic dose (30 mg./kg.) takes about 5 minutes to produce its action. The median lethal dose (60 mg./kg.) actually takes nearly 2 hours, but this does not appear to be a true measure of the rate of action of the drug since the duration of sleep in the case of the survivors at this dosage is about the same time. This recovery indicates that a considerable clearance of nembutal occurs in 2 hours and hence the occurrence of death at this time is evidently a delayed death due to some secondary effect such as prolonged partial anoxæmia during the deep narcosis. A dose of about twice the M.L.D. produces death in 10 minutes. This is far more rapid action than that occurring with the delayed deaths, but considerably slower than the action of evipan.

The delay in the rate of action is best seen in the case of the long-acting drug sodium barbitone. In this case (Table III.) a lethal dose (500 mg./kg.) only produced hypnosis after 5 minutes and the medium

Table II.—Action of Intravenous Nembutal on Mice.

I.V. dose, mg./kg.	Lethal effects.			Hypnotic effects.		
	No. injected.	Incidence death.	Mean time till death in min.	Incidence sleep.	Mean time before onset of sleep in min.	Duration of sleep in min.
200	3	3/3	1·6			
150	3	3/3	7			
125	3	2/3	10	1	..	140
100	3	3/3	45			
80	3	2/3	100	1	..	120
60	3	2/3	100	1	..	120
45	4	0/4	..	4/4	1 to 2	40
40	7	0/7	..	7/7	2 to 3	30
36	8	0/8	..	8/8	3 to 5	25
30	3	0/3	..	2/3	5	21
25	4	0/4	..	1/4	5	30

hypnotic dose (250 mg./kg.) took 22 minutes to produce hypnosis. *Delayed deaths after many hours occurred over a considerable range of* dosage.

Table III.—Action of Intravenous Sodium Barbitone on Mice.

Dose, mg./kg.	Latent period.	Effects.
250	Slept after 22 min.	Slept for 30 min.
400	,, 6 ,,	,, 6 hours.
500	,, 5 ,,	Slept overnight, found dead next morning.
600	,, 3 ,,	,, ,, ,,
800	,, 3 ,,	,, ,, ,,
1200	Slept at once.	,, ,, ,,
2000	,,	Died in 30 min.
3000	Died in 1 min.	

Discussion.

Intravenous injection into mice forms a convenient method for demonstrating the fact that the short-acting and long-acting hypnotics differ not only as regards their duration of action, but also as regards rate of action. The four barbiturates considered form a series both as regards duration of action and as regards rate of action. The slow action of sodium barbitone might be due to the fact that it is a much

feebler narcotic than sodium evipan, but this explanation will not hold in the case of nembutal, the activity of which is similar to that of sodium evipan. A consideration of the times at which deaths occur indicates that there are two classes, the immediate deaths due to the direct action of the drug and the delayed deaths due to some indirect action. The latter class occur most frequently with the long-acting barbiturates but sometimes occur with the short-acting barbiturates.

SUMMARY.

1. The rate of detoxication of quick-acting barbiturates in mice can be determined by the method of continuous intravenous infusion into the tail vein following a suitable initial dose.

2. The rate of detoxication of sodium evipan was found to lie between 1/28 to 1/35 of the quantity present per minute, and of pentothal sodium between 1/40 and 1/50.

3. Barbiturates such as barbitone, which produce a prolonged action, also act much more slowly than the labile barbiturates and hence the method described above does not give satisfactory results.

REFERENCE.

DAS, S. C., and RAVENTÓS, J. (1939). *Quart. J. exp. Physiol.* 29, 343.

THE SECRETION OF URINE IN RABBITS DURING EXPERIMENTAL SALT DEFICIENCY. By B. M. Wilkinson and R. A. McCance. From the Belgrave Hospital for Children, London, and The Department of Medicine, Cambridge.

(*Received for publication* 17*th April* 1940.)

The work of the nineteenth and early twentieth centuries established the fact that salt deficiency and azotæmia tended to occur together. As the experimental evidence accumulated and as the blood chemistry of one disease after another was investigated it became probable that there was a causal connection, and in 1928 Blum and his associates [Blum *et al.*, 1928, 1929] put forward a theory to explain how this was brought about. Although this theory seems to have been based upon a misconception, the suggestion was an important one for two reasons. Firstly, it drew attention to the possibility that a number of isolated phenomena might have one common pathology, and, secondly, it stimulated a great deal of observation and experimental work.

It has now been shown that salt deficiency, experimentally induced both in animals [Bilbao and Grabar, 1929; Glass, 1932; Michelsen, 1933; Harrison and Darrow, 1939] and man [McCance and Widdowson, 1937], causes a rise in the blood urea. In man the rise was attributed (*a*) to a reduction in the glomerular filtration rate, (*b*) to an increased re-absorption of urea (Blum's 1928–29 theory), and (*c*) to the forced breakdown of body proteins. All three were demonstrated by experiment to accompany a deficiency, and the first was thought to be by far the most important. The fall in glomerular filtration rate, and also the increased re-absorption of urea, have been confirmed by Harrison and Darrow [1939] as part of the syndrome of salt deficiency in dogs. McCance and Widdowson [1937] showed that the fall in glomerular filtration rate brought about by salt deficiency in man was not due to a fall in arterial blood-pressure (Glatzel [1937] misquoted McCance as having said that the arterial blood-pressure fell), and considered that it might be due to anhydræmia. This could reduce the glomerular filtration rate in a number of ways, *e.g.* by raising the colloidal osmotic pressure of the plasma, by reducing the volume of the circulating blood so that the usual number of glomeruli were not filled, and by increasing the viscosity of the blood and so reducing the circulation rate. McCance and Widdowson, however, made the reservation that there might be

feebler narcotic than sodium evipan, but this explanation will not hold in the case of nembutal, the activity of which is similar to that of sodium evipan. A consideration of the times at which deaths occur indicates that there are two classes, the immediate deaths due to the direct action of the drug and the delayed deaths due to some indirect action. The latter class occur most frequently with the long-acting barbiturates but sometimes occur with the short-acting barbiturates.

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60–90 minutes before the clearances were determined. The animals' blood-pressures were taken by Grant and Rothschild's [1934] method. The red blood corpuscles were counted in a Thoma chamber and the hæmoglobin was determined by Haldane's method (100 per cent. of hæmoglobin = 13·8 g./100 c.c. of blood). Creatinine was determined by Folin's method [Matthews, 1930] and urea by Lee and Widdowson's method [1937].

Results.

Nitrogen Intakes and Nitrogen Balances.

Before this investigation began it was known that salt deficiency in rabbits raised the blood urea [Bilbao and Grabar, 1929; Michelsen, 1933], and the experiments on man had made it probable that this was mainly due to a reduced glomerular filtration rate. Since, however, one of the main objects of the investigation was to make a study of the rabbit which would be comparable with that of man, all the possible causes of a high blood urea, which had been investigated in the latter, were explored. It was at once evident that the high blood ureas were not due to a high protein intake since the rabbits ate very little during the production and duration of salt deficiency. Three balance experiments

Table I.—Nitrogen Balance of Rabbit I.

Day.	N Intake, g.	N Output, g.	Balance, g.	Weight, kg.	Blood urea, mg./100 c.c.
1 *d*	1·28	2·40	−1·12	3·45	29
2 *d*	0·82	2·61	−1·79	3·25	
3 *d*	0·73	0·70	+0·03	3·17	
4	0·32	0·85	−0·53	3·15	
5 *d*	0·75	0·10	+0·65		32
6 *d*	1·47	2·61	−1·14	3·15	46
7 *d*	1·44	0·47	+0·97	3·15	
8 *d*	0·41	2·39	−1·98		56
9 *d*	0·39	0·96	−0·57	3·17	60
10	0·51	1·43	−0·92	3·12	73
Total loss			6·40	0·33	
Recovery period—salt administered.					
1	1·61	0·30	+1·31	3·20	
2	1·59	0·68	+0·91		
3	1·66	1·74	−0·08	3·26	
4	1·49	0·98	+0·51	3·26	27
Total gain			2·65	0·14	

The salt deficiency was induced by diuretin, which was given on the days marked with a "*d*."

some unknown and hitherto unsuspected cause which was primarily responsible. This they did because uncompensated alkalosis in man may produce a profound fall of glomerular filtration rate without any anhydræmia and often without any fall of blood-pressure. The subject of salt deficiency and its attendant azotæmia has been reviewed from slightly different aspects by Glatzel [1937], Kerpel-Fronius [1936], Mach [1937, 1938], and McCance [1936].

The present investigation was undertaken to study the secretion of urine during salt deficiency in another species and particularly to study the cause of the fall in glomerular filtration rate, if such should be discovered. The rabbit was chosen (*a*) because it can be made salt deficient in two ways, neither of which grossly interferes with the function of the gastro-intestinal tract; (*b*) because it has a kidney which is functionally rather different from that of the dog or man [Kaplan and Smith, 1935]; (*c*) because Michelsen's [1933] work had suggested that it might be possible in this animal to induce salt deficiency without anhydræmia.

PRESENT INVESTIGATION.

Methods.

Adult male rabbits weighing about 3 kg. have been used throughout. Salt deficiency was induced either by the administration of diuretin [Bilbao and Grabar, 1929], or by injecting a 5 per cent. solution of glucose into the peritoneal cavity and draining off as much of the fluid as possible 3 hours later [Gilman, 1934]. Two injections were never made within 24 hours of each other. Water was administered through a No. 9 rubber catheter passed into the stomach. Urine was collected from the bladder by a No. 3 rubber catheter. For this operation the rabbit was supported in a sitting position and the infant catheter, lubricated with paraffin, was passed into the urethra in a slightly downward direction for about 2 cm. The catheter and penis were then depressed and the former passed on into the bladder. Firm abdominal pressure was used to empty the viscus completely and it was not washed out by injecting water or air. In carrying out clearances at high rates of urine flow the experimental periods varied from 15 to 60 minutes according to the expected volume. Blood was collected as required from the marginal ear vein. For clearance tests it was withdrawn exactly in the middle of each period. The blood was treated with minimal quantities of oxalate and centrifuged as quickly as possible. Plasma was used for all chemical determinations. Intravenous injections were made into the marginal vein of the ear. The creatinine clearance was taken as a measure of the glomerular filtration rate [Kaplan and Smith, 1935]. The plasma creatinine was raised by injecting about 1 g. of creatinine subcutaneously as a 5 per cent. solution

60–90 minutes before the clearances were determined. The animals' blood-pressures were taken by Grant and Rothschild's [1934] method. The red blood corpuscles were counted in a Thoma chamber and the hæmoglobin was determined by Haldane's method (100 per cent. of hæmoglobin = 13·8 g./100 c.c. of blood). Creatinine was determined by Folin's method [Matthews, 1930] and urea by Lee and Widdowson's method [1937].

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were carried out. The results of one are given in Table I. The others were similar except that in them the intakes and outputs were not measured during the recovery phase. The small N intake and the negative balance are clearly shown during the deprivation period and the increased intake and positive balance during the recovery period. The rabbit's normal N intake may be gauged from the intake during recovery. If an allowance had been made for the fluctuations in the blood urea the negative and positive balances shown in Table I. would both have been larger [McCance, 1935–36]. These results are very like those obtained on man [McCance, 1935–36]. They differ mainly in that the humans managed to maintain their food intakes almost within normal limits while they were salt deficient. This, however, did not prevent them being in negative nitrogen balance. Compare also for this Michelsen's [1933] observations.

24-*hour Urea Clearances.*

For these tests the animals were kept in metabolism cages and the urines were allowed to collect for 24 hours under toluene. At the end of the 24-hour periods the rabbits were lifted and induced to empty their bladders by firm abdominal pressure. Eight experiments, each lasting 3–5 days, were carried out on 6 normal rabbits and the effects of salt deficiency were studied in 7 animals by means of 10 experiments. The animals were given a weighed ration of cabbage every day, usually 150–200 g., and this was always eaten. They were in all but a few experiments allowed free access to water, and in some additional water was administered by stomach tube. In Tables II. and III. the heading "Total fluid intake" includes not only the water taken as such but also

TABLE II.—AVERAGE DAILY WATER BALANCES, UREA CLEARANCES, ETC., OF NORMAL RABBITS.

Rabbit.	Days under observation.	Total fluid intake, c.c./day	Urine, c.c./day.	Urine flow, c.c./sq. m./min.	Blood urea, mg./100 c.c.	Urea clearance, c.c./sq. m./min.	Rise or fall of percentage of hæmoglobin.
J	4	155	151	0·528	32·5	27·3	..
R	4	204	217	0·725	22·0	27·0	..
P	3	196	220	0·864	29·0	20·9	..
E	3	186	199	0·765	39·0	18·3	..
I	4	297	272	0·900	33·5	21·8	..
E *r*	4	90	53	0·200	33·0	19·5	0
D *f*	3	234	240	0·735	23·0	..	+3
R *f*	3	255	148	0·500	17·5	23·8	−9

r indicates that the fluid intake was restricted.
experimentally raised.

the water in the cabbage, and, in those animals being made deficient by glucose, the fluid which could not be recovered from the peritoneal cavity. The blood used for estimating urea was taken as nearly as possible half-way through the period over which urine was being collected. When the blood ureas were rising in the deficient animals considerable inaccuracies may have attended the determination of the clearances, but it is thought that these can be ignored since the changes in the clearances were so gross. Blood for hæmoglobin was taken just before glucose was injected into the peritoneal cavity.

TABLE III.—WATER BALANCES, CLEARANCES, ETC., IN SALT DEFICIENT RABBITS.

Rabbit.	Day.	Total fluid intake, c.c./day.	Urine, c.c./day.	Urine flow, c.c./sq. m./min.	Blood urea, mg./100 c.c.	Urea clearance, c.c./sq. m./min.	Hæmoglobin, per cent.
E	1 *g*	147	188	0·74	37	20·20	77
	2 *g*	131	87	0·34	87	3·05	78
	3	102	28	0·11	133	0·28	75
E *f*	1 *g*	235					80
	2 *g*	315	373	1·42	28	33·10	82
	3 *g*	312	348	1·33	25	20·30	77
	4	240	162	0·62	40	9·00	66
R *r*	1 *g*	90					73
	2 *g*	110	70	0·23	25	13·50	78
	3 *g*	130	44	0·14	84	1·86	74
	4	100	30	0·09	165	0·29	73
R *f*	1 *g*	312					73
	2 *g*	229	345	1·17	23	30·30	71
	3 *g*	245	235	0·81	28	14·20	69
	4 *g*	312	34	0·12	68	1·00	64
	5	132	95	0·32	141	1·99	58

r indicates that the fluid intake was restricted, *f* that it was artifically raised. On days marked with a *g* glucose was administered intraperitoneally.

In normal rabbits the intake of fluids varied considerably from day to day and was not always accompanied by a corresponding change in output. It is probable that a rabbit's water metabolism is much less sensitively adjusted than a man's or a dog's. The normal blood ureas did not vary much from day to day, nor did the urea clearances, although fluctuations of 14 to 36 c.c./sq. m./min. were observed in one animal. In Table II. will be found the average results for each of the normal rabbits. Table III. shows experiments illustrating the effect of progressive salt deficiency on the blood urea, urine volume, and urea clearance. The animals were made deficient by injecting glucose

intraperitoneally, and the experimental days on which this was done are marked with a *g*. The normal results for these animals are given in Table II. It will be observed that as salt deficiency developed, (*a*) excluding rabbit E*f*, which was quite exceptional, there was a tendency for the urine volume to fall, even when the animal had free access to water. This tendency was more clearly shown in some of the experiments which have not been quoted. (*b*) The blood ureas invariably rose. (*c*) The clearances fell to an extremely low figure in three of the experiments. In the fourth the fall was not so great and was accompanied by the smallest rise in blood urea observed in all these experiments. The explanation of both these findings probably lies in the fact that the water intake was artificially raised, and this in turn increased the flow of urine and urea clearance (*vide infra*). Very different results were obtained when this animal was allowed to regulate its own consumption of water and these are also shown in Table III. (*d*) As judged by hæmoglobin measurements there was never any appreciable anhydræmia 24 hours after a paracentesis and the administration of extra water caused some hydræmia. (*e*) The changes in weight were negligible. When all the experiments were considered together it was evident that, although there was no anhydræmia, an oliguria was the natural outcome of salt deficiency, and these facts must indicate abnormal retention of water. In man oligurias of this degree were not observed during salt deficiency, but at times there was an increase of body weight which must have been due to a retention of water. This phenomenon therefore is common to both species.

Creatinine and Urea Clearances at High Minute Volumes.

Kaplan and Smith [1935] showed that in the rabbit the glomerular filtration rate varied with the minute volume of the urine. This has been amply confirmed in the present investigation at high rates of urine flow, and therefore it has been necessary to compare the clearances of the normal with those of the deficient animals at similar rates of flow. In order to do this normal rabbits were given water by stomach tube, catheterised at appropriate intervals and their clearances determined as described earlier in this paper. The same animals were then made salt deficient either by glucose or by diuretin, and, 24 hours after the last paracentesis, attempts were made to redetermine the clearances at comparable minute volumes. This, however, was never very easy to do because of the difficulty of promoting an adequate diuresis. More water had to be given, and given at an earlier time than to the normals, and even then some deficient animals never produced even a moderately high rate of urine flow. No volumes over 5·4 c.c./sq. m./min. were obtained, whereas volumes up to 8 c.c./sq. m./min. were sometimes recorded in the normal animals. Proceeding along the lines described,

curves relating clearances and minute volumes were secured from the normal and deficient animals. The averaged results are compared in fig. 1 in which each point represents the mean of 10–14 individual determinations. Data obtained from 3 individual animals are given in fig. 2. It will be seen that the clearances of both creatinine and urea were reduced by salt deficiency, and that the higher the level of urea in the blood the lower the clearances tended to be (fig. 2, rabbits *y* and *z*).

The ratios of the urea/creatinine clearances in normal and salt deficient animals at different urine flows were as follows:—

Normal flows, c.c./sq. m./min., 0·97, 2·07, 2·92, 4·02, 5·10, 5·92, 7·20, 8·05.
Normal U/C clearance ratios, 0·39, 0·47, 0·44, 0·46, 0·51, 0·44, 0·47, 0·47.
Salt-deficient urine flows, c.c./sq. m./min., 1·00, 2·04, 3·10, 4·22, 4·91.
Salt-deficient U/C clearance ratios, 0·43, 0·45, 0·47, 0·48, 0·50.

The normal ratios average 0·46 and the deficient 0·47. The agreement is a close one, and shows that at these urine flows there was no additional re-absorption of urea by the tubules of the salt deficient animals.

There are two other ways of looking at the results, summarised in fig. 1. Both are instructive. In the first place, at equal minute volumes the deficient animals had lower U/B ratios than the normal ones. In the second, at equal glomerular filtration rates, the deficient animals had larger urine volumes, *i.e.* they re-absorbed less water from their glomerular filtrates.

Creatinine and Urea Clearances at Low Minute Volumes.

Kaplan and Smith [1935] found that the clearances of creatinine and urea continued to fall as the minute volumes fell below 1. This was the effect to be expected from a consideration of the minute volume-clearance curves between 1 and 6 c.c./sq. m./min. In the present investigation the clearances during salt deficiency have behaved in this way, but for some reason the clearances of the normal animals have not (fig. 1). In the 24-hour clearances, moreover, at urine flows averaging 0·922 c.c./sq. m./min. the urea clearances averaged 22·2 c.c./sq. m./min. and at flows averaging 0·535 c.c./sq. m./min., 25 c.c./sq. m./min. The reason for these rather unexpected findings may be technical in origin, but there may be some unknown factor complicating the issue, and this seems the more probable explanation. There is, however, good reason to believe that the very low minute volumes encountered during the studies of 24-hour urea clearances in salt deficient animals must have been associated with very low creatinine clearances (glomerular filtration rates) and that these were in themselves almost enough to account for the high blood ureas.

intraperitoneally, and the experimental days on which this was done are marked with a *g*. The normal results for these animals are given in Table II. It will be observed that as salt deficiency developed, (*a*) excluding rabbit E *f*, which was quite exceptional, there was a tendency for the urine volume to fall, even when the animal had free access to water. This tendency was more clearly shown in some of the experiments which have not been quoted. (*b*) The blood ureas invariably rose. (*c*) The clearances fell to an extremely low figure in three of the experiments. In the fourth the fall was not so great and was accompanied by the smallest rise in blood urea observed in all these experiments. The explanation of both these findings probably lies in the fact that the water intake was artificially raised, and this in turn increased the flow of urine and urea clearance (*vide infra*). Very different results were obtained when this animal was allowed to regulate its own consumption of water and these are also shown in Table III. (*d*) As judged by hæmoglobin measurements there was never any appreciable anhydræmia 24 hours after a paracentesis and the administration of extra water caused some hydræmia. (*e*) The changes in weight were negligible. When all the experiments were considered together it was evident that, although there was no anhydræmia, an oliguria was the natural outcome of salt deficiency, and these facts must indicate abnormal retention of water. In man oligurias of this degree were not observed during salt deficiency, but at times there was an increase of body weight which must have been due to a retention of water. This phenomenon therefore is common to both species.

Creatinine and Urea Clearances at High Minute Volumes.

Kaplan and Smith [1935] showed that in the rabbit the glomerular filtration rate varied with the minute volume of the urine. This has been amply confirmed in the present investigation at high rates of urine flow, and therefore it has been necessary to compare the clearances of the normal with those of the deficient animals at similar rates of flow. In order to do this normal rabbits were given water by stomach tube, catheterised at appropriate intervals and their clearances determined as described earlier in this paper. The same animals were then made salt deficient either by glucose or by diuretin, and, 24 hours after the last paracentesis, attempts were made to redetermine the clearances at comparable minute volumes. This, however, was never very easy to do because of the difficulty of promoting an adequate diuresis. More water had to be given, and given at an earlier time than to the normals, and even then some deficient animals never produced even a moderately high rate of urine flow. No volumes over 5·4 c.c./sq. m./min. were obtained, whereas volumes up to 8 c.c./sq. m./min. were sometimes recorded in the normal animals. Proceeding along the lines described,

curves relating clearances and minute volumes were secured from the normal and deficient animals. The averaged results are compared in fig. 1 in which each point represents the mean of 10–14 individual determinations. Data obtained from 3 individual animals are given in fig. 2. It will be seen that the clearances of both creatinine and urea were reduced by salt deficiency, and that the higher the level of urea in the blood the lower the clearances tended to be (fig. 2, rabbits y and z).

The ratios of the urea/creatinine clearances in normal and salt deficient animals at different urine flows were as follows:—

Normal flows, c.c./sq. m./min., 0·97, 2·07, 2·92, 4·02, 5·10, 5·92, 7·20, 8·05.
Normal U/C clearance ratios, 0·39, 0·47, 0·44, 0·46, 0·51, 0·44, 0·47, 0·47.
Salt-deficient urine flows, c.c./sq. m./min., 1·00, 2·04, 3·10, 4·22, 4·91.
Salt-deficient U/C clearance ratios, 0·43, 0·45, 0·47, 0·48, 0·50.

The normal ratios average 0·46 and the deficient 0·47. The agreement is a close one, and shows that at these urine flows there was no additional re-absorption of urea by the tubules of the salt deficient animals.

There are two other ways of looking at the results, summarised in fig. 1. Both are instructive. In the first place, at equal minute volumes the deficient animals had lower U/B ratios than the normal ones. In the second, at equal glomerular filtration rates, the deficient animals had larger urine volumes, *i.e.* they re-absorbed less water from their glomerular filtrates.

Creatinine and Urea Clearances at Low Minute Volumes.

Kaplan and Smith [1935] found that the clearances of creatinine and urea continued to fall as the minute volumes fell below 1. This was the effect to be expected from a consideration of the minute volume-clearance curves between 1 and 6 c.c./sq. m./min. In the present investigation the clearances during salt deficiency have behaved in this way, but for some reason the clearances of the normal animals have not (fig. 1). In the 24-hour clearances, moreover, at urine flows averaging 0·922 c.c./sq. m./min. the urea clearances averaged 22·2 c.c./sq. m./min. and at flows averaging 0·535 c.c./sq. m./min., 25 c.c./sq. m./min. The reason for these rather unexpected findings may be technical in origin, but there may be some unknown factor complicating the issue, and this seems the more probable explanation. There is, however, good reason to believe that the very low minute volumes encountered during the studies of 24-hour urea clearances in salt deficient animals must have been associated with very low creatinine clearances (glomerular filtration rates) and that these were in themselves almost enough to account for the high blood ureas.

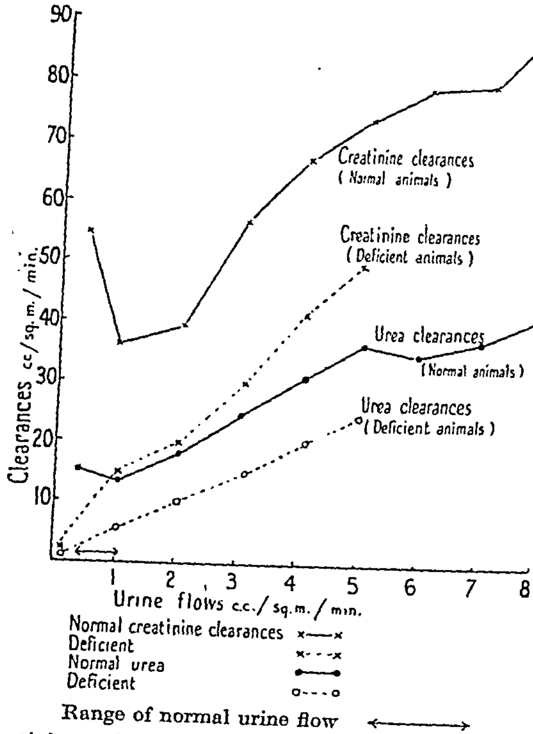
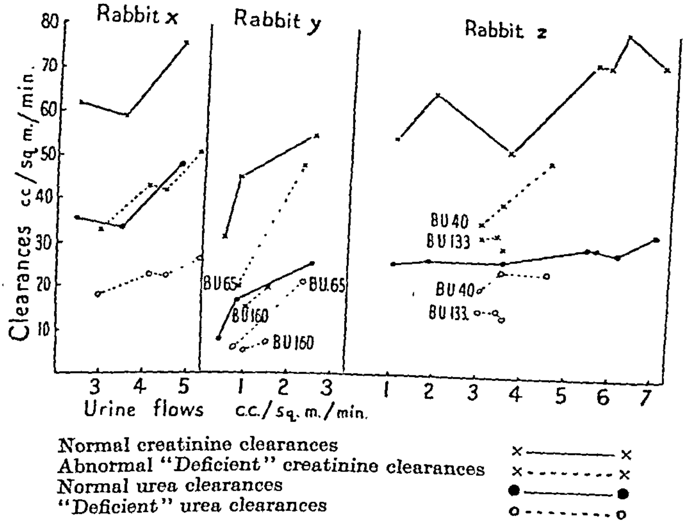


FIG. 1.—Creatinine and urea clearances of normal and salt deficient rabbits at high rates of urine flow (averaged results).



B.U. = level of blood urea at which the set of clearances was carried out.

FIG. 2.—The effect of salt deficiency on the clearances of individual rabbits.

Defective Diuresis after Forced Water in Salt Deficient Rabbits.

Reference has already been made to the difficulty of obtaining high rates of urine flow during salt deficiency. This is a phenomenon which has been observed in man [Baldes and Smirk, 1934; McCance, 1936; McCance and Widdowson, 1937] and was a very characteristic feature of the human experimental work. It is an aspect of salt deficiency which may be connected with the fall in glomerular filtration rate, although there is no direct evidence for this. Some quantitative experiments have been carried out on the rabbits, one of which is illustrated in fig. 3, A. It will be seen that salt deficiency prevented the

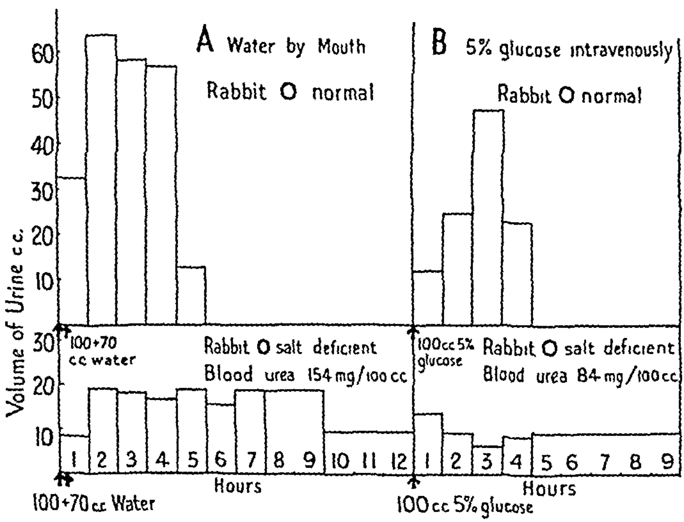


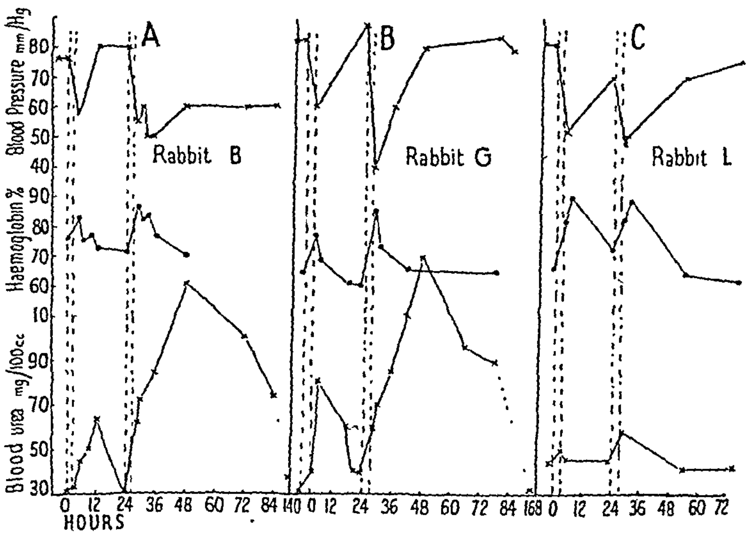
FIG. 3.—The inhibiting effect of salt deficiency upon the production of a diuresis.

normal diuresis after giving the water by mouth. This is just what happens in man. Most of the water may ultimately be excreted at a slower rate, but the fine adjustment between intake and output has been destroyed. Fig. 3, B, shows that the course of events was similar after fluid (5 per cent. glucose) had been given intravenously, so that delayed absorption from the intestine is not the cause of the slow diuresis during salt deficiency.

Cause of the Fall in Glomerular Filtration Rate.

The changes in function induced by salt deficiency are very reminiscent of those which McCance and Widdowson [1937] obtained in normal men, and it is clear that the pathology is essentially the same in both species. This is satisfactory since the reasons for the fall in glomerular filtration rate can be analysed more fully in the rabbit than they can in man. The usual explanations are (1) anhydræmia [Gömöri

et al., 1939], (2) a low blood-pressure. Experiments have been carried out to study the relationship of both of these to the rise and level of blood urea in the rabbits. The animals were made salt deficient by intraperitoneal glucose and their blood-pressures, percentages of hæmoglobin and blood ureas were followed. In these short experiments a change in the percentage of hæmoglobin was taken to indicate a change in the volume of the circulating plasma and to be a permissible index of the degree of hydræmia. The findings in three experiments are shown in fig. 4, A, B, and C. The time relationships are important. It will be



The glucose solution was in the peritoneal cavity for the intervals of time between the dotted vertical lines.

FIG. 4.—Course of the blood pressure, hæmoglobin, and blood urea during the production of salt deficiency by intraperitoneal glucose.

noted that in each experiment the hæmoglobin rose and the blood-pressure fell immediately after the first paracentesis [Gilman, 1934]. If, subsequently, determinations were made at frequent intervals the hæmoglobin was always found to return quite quickly to normal. The blood-pressure findings were more variable, but after 24 hours the readings were always near the normal, generally slightly below—but see fig. 4, A. The second intraperitoneal administration of glucose and the subsequent paracentesis had similar but more pronounced effects on the hæmoglobin and the blood-pressure. After a further 24 hours had passed, however, the hæmoglobin was always normal and the pressure normal or approaching normal. The pressure curve in fig. 4, A, is again rather exceptional in this respect. The fluctuations in blood urea were very variable. It was usual to have a large rise after the second paracentesis, which reached its peak 24 or more hours later.

Sometimes the urea rose for 48 hours and to levels above 200 mg./100 c.c. Sometimes it scarcely changed at all (fig. 4, C). The following considerations suggest that the changes in hydræmia and blood-pressure were not in themselves enough to account for the renal disorganisation as judged by the high ureas and the reduced clearances. (1) There was no correspondence between the fall of pressure, rise of hæmoglobin, and the rise of blood urea. In the experiment shown in fig. 4, A, for example, the blood urea finally fell at a blood-pressure at which it had initially risen. In another experiment (fig. 4, C) the changes in blood-pressure and hæmoglobin were quite as great or greater than they were in the experiment shown in fig. 4, A, yet there was no more than a slight and transient rise in the blood urea. (2) Whatever the immediate effects of paracentesis on the hæmoglobin and blood-pressure, the former was always and the latter almost always within normal limits 24–30 hours after the second paracentesis. Yet this was the time the blood ureas were often at their highest (fig. 4, B) and the clearances were certainly not normal, for (*a*) the present work has gone to show that in rabbits clearances tend to vary inversely as the blood ureas (fig. 2). (*b*) The clearances at high rates of urine flow (*vide supra*) were always tested 24–30 hours after a paracentesis. They were always subnormal when the rabbits were salt deficient and their blood ureas high. Anhydræmia, therefore, and hypotension may have depressed the glomerular filtration rates for some hours after each paracentesis, but it is improbable that either were major causes of low glomerular filtration rates 24 hours after a paracentesis. To Gömöri *et al.* [1939] anhydræmia (exsiccosis) is the one important cause of renal dysfunction in salt deficiency. This is probably due to the fact that these authors have always produced their salt deficiencies by pyloric ligature. This method would inevitably have led to an "exsiccosis." Gömöri, moreover, used cats. In man, and probably also in cats and dogs, anhydræmia inevitably accompanies salt deficiency: in rabbits, however, see Table III., this is not necessarily the case, for the anhydræmia can be overcome by giving fluids. If, therefore, Gömöri had worked on rabbits, and had used several methods of producing salt deficiency, he might have reached other conclusions.

It may be that in salt deficiency the blood-flow through the kidney is reduced by some local vasomotor mechanism. There is no evidence at present to support this, but possibly the evidence could be obtained. It may be that the glomerular filtration rate is reduced by some metabolic abnormality in the glomerular membranes or by a dilatation of the efferent glomerular arterioles. These are mere conjectures. The present results, however, suggest that there are local as well as general reasons for the fall in glomerular filtration during salt deficiency, and that some cause for the fall will be found in the kidney itself.

Causes of the High Blood Ureas.

When one turns to a consideration of the causes of the high blood ureas it is clear that these are (1) a reduced glomerular filtration rate at all urine flows (rabbit and man). (2) An extreme oliguria (in the rabbit) at normal fluid intakes. This oliguria, which is due to abnormal water retention, can be prevented by raising the fluid intake to a sufficiently high level. It is not obvious in man, although man also shows abnormal water retention when he is salt deficient and fails to excrete water normally under test. (3) Excessive breakdown of body proteins—probably an unimportant cause in both species.

Summary.

(1) Salt deficiency was induced experimentally in the rabbit (*a*) by administering diuretin by mouth to animals on a low salt diet, (*b*) by injecting glucose solutions intraperitoneally and withdrawing the fluid some hours later by paracentesis.

(2) Salt deficiency so induced led to

(*a*) A negative N balance and possibly an excessive breakdown of body proteins.

(*b*) An abnormal water balance characterised by a failure to excrete water normally under test and by an extreme oliguria at normal fluid intakes.

(*c*) A reduced glomerular filtration rate at all urine flows which cannot be satisfactorily explained by anhydræmia or a fall of blood-pressure and the cause of which probably lies in the kidney itself.

(*a*), (*b*), and (*c*) acting together lead to very high blood ureas.

(3) Salt deficiency in the rabbit produces the same signs of renal disorganisation as it does in man, and the pathology must be essentially the same in both species.

Much of this work was done at the Medical School of King's College Hospital, London, and the authors wish to acknowledge with thanks the facilities which they were given there.

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EXPERIMENTAL HEART-FAILURE IN RABBITS. By T. G. Armstrong. From the Department of Pathology, Cambridge University.

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For some time previous to the war I was engaged in an attempt to produce an experimental constrictive pericarditis in rabbits. The method employed followed closely that of Beck [1929, 1930], who irrigated the pericardium of dogs with Dakin's fluid. Owing to the smallness of the animal, however, and the delicacy of the pericardium, irrigation was not found feasible, and a simple injection of a small quantity of the solution was made into the pericardium. The results were surprising, for the animals, with very few exceptions if a strong enough solution was used, died of massive pleural effusions within fourteen days of operation. It soon became evident that the condition produced in these animals, although not constrictive pericarditis, bore great similarity to the results reported by Dible and Lynch [1938]. They observed the same phenomenon after the injection of small quantities of tincture of iodine into the pericardium of rabbits. They attributed the effusions to two processes: firstly, to an increase in the permeability of the pleural capillaries as a result of the aseptic inflammation of the mediastinum; and secondly, to an obstruction to the egress of fluid from the pleura along the mediastinal lymphatics. They were clearly of the opinion that failure of the heart played no part in the production of the hydrothorax, and deliberately rejected it as a cause, after mature consideration. As my own work was primarily concerned with the heart, my attention was focussed on this organ and its dysfunction. Working on these lines, my investigation of the causes of the hydrothorax yielded results very different from those of Dible and Lynch.

Method Employed.

The approach to the pericardium was exactly similar to that employed by Dible and Lynch. A mid-line incision was made over the sternum, and the pectoral muscle was separated from the sternum and costal cartilages and reflected laterally. A thin sheet of muscle—the pectoralis minor—was then separated and retracted laterally, leaving the ribs and costal cartilages entirely bare. Fine-pointed scissors were used to cut the 3rd, 4th, and 5th costal cartilages at their extreme

anterior ends at their attachment to the sternum. It is of great importance that they should be separated at this site, for if the cut is made too far laterally the internal mammary artery may be divided, or a pneumothorax may ensue. When the costal cartilages had been freed, a vertical cut was made with the scissors through the fibres of the transversus thoracis muscle and the fascia posterior to it. Considerable attention must be paid to this step, for the fascia behind the transversus thoracis muscle is liable to be mistaken for the pericardium, in which case the injection is made into the anterior mediastinal tissues instead of into the pericardium itself, and no result will follow the operation. After division of this fascia the heart and pericardium will be seen overlaid by the thymus and a small quantity of fatty tissue. These tissues should then be retracted headwards after separation from the pericardium with a blunt dissector. When this is done, a clear and wide exposure of the pericardium and heart is obtained. The injection is best made with a small needle, the last $\frac{1}{3}$ of an inch of which has been bent to an angle of about 130°.

The substances used for injection were three in number:

1. Tincture iodi mitis—0·2 c.c.
2. Dakin's solution. Full strength. 0·2 c.c. to 0·5 c.c. Owing to the rapid deterioration of this solution it is important that only freshly prepared Dakin's fluid should be used.
3. * Gelatine, calcium chloride, and acriflavine solution, 0·5 c.c. to 1·0 c.c.

The effects of these three solutions injected into the pericardium were practically the same. No further mention will be made of the gelatine-calcium chloride solution: it is quoted only to confirm Dible and Lynch's finding that a variety of irritants produce the same result.

Immediately after the injection is made into the pericardium, the heart is seen to dilate, and the rate slows until only a few feeble contractions are taking place. Very soon the rate and force of the contractions return, but the size of the heart remains larger than normal, at least for the short time until the chest is closed. This acute dilatation of the heart is most marked when Dakin's fluid is injected, but is well seen, though to a less extent, after the injection of tincture of iodine.

Mention must also be made here of certain other technical procedures which were used before and after operation in these animals. In a small series of cases the size of the heart was investigated by radiography before and after operation. The method adopted was to tie the animal down on a long plywood board, reinforced by battens at the sides and ends. The fore-paws were held firm by a string which passed

* Composition gelatine 5 per cent., calcium chloride 5 per cent., in 1/2000 acriflavine in normal saline. This is recommended by Hunter for producing calcification and hyaline fibrosis in the lung.

over two cleats anteriorly, and the hind legs were secured by a similar process behind. The animal was prevented from rotating or moving laterally by two padded flanges at right angles to the main plane of the apparatus. These flanges formed a gutter about four inches deep into which the thorax of the animal fitted. In this way standard conditions were obtained, and the animal was always in the same position for the series of radiographs which were taken. The film was placed between the animal's chest and the plywood. The animals were all radiographed in the vertical position, as it was found that a clearer view of the heart was obtained in this way than when the horizontal position was used.

In a further small series of cases arterial blood-pressures were taken before and after operation. The method employed was that of compressing the arteries of the ear by a rubber capsule connected to a mercury manometer. The systolic pressure was taken as the lowest point at which the column of blood was broken. Readings were always taken after the animals had been confined in a warm box until the ear vessels showed a maximal dilatation. In the final acute experiment the systolic and diastolic blood-pressures were taken by a direct method, using a cannula and a Wigger's capsule.

Venous pressures were taken in all cases by the direct method. A long cannula was introduced into the jugular vein and passed down to the base of the heart. The cannula was attached to a manometer containing citrate solution. Readings were taken when it was seen that there was a free movement of the column of fluid in the manometer. In most cases the pressures were taken at the base of the heart, but in a small series pressures were taken, in addition, from the jugular veins themselves and from other veins, including the portal. In all cases the zero reading was taken from the level of the base of the heart, the position of which was directly measured in each animal.

Proof of the Identity of the Effects of Dakin's Fluid and Tincture of Iodine.

In the first instance, it was the post-mortem appearances which suggested that the condition produced after the injection of Dakin's fluid was identical with that produced by iodine. As the morbid anatomical findings were essentially the same, a general description of both will be given, and two short protocols of representative experiments will be appended.

Immediately after recovery from the anæsthetic the animals appeared well, and by the following day the appetite was normal. The first sign of trouble was usually a failure of appetite, but this seldom ensued before the 6th or 7th day after operation. Even so, the animals appeared well until they suddenly became dyspnœic at some time

between the seventh and fourteenth day. The onset of marked dyspnœa was seldom survived for longer than 24 hours. Dyspnœa was obvious in degree, and the animals would be seen with their heads thrown back and the alæ nasi distending with each laboured breath. Even at this stage it was seldom possible to demonstrate effusions with the stethoscope or by percussion. Often, but not invariably, œdema of the scrotum and abdominal wall and of the subcutaneous tissue of the chest might be demonstrated. Death ensued rapidly and took place quite suddenly. If the effusions were tapped they rapidly recurred, and it was impossible to stave off a fatal termination by this means.

Post-mortem showed frequently, but not always, œdema of the dependent parts, particularly of the scrotum and abdominal wall; in bucks, œdema always appeared first in the scrotum.

On opening the chest, 20 to 50 c.c. of fluid, sometimes blood-stained, were usually found in each pleural cavity. Often there was a dense network of fibrin covering the pleural surfaces, particularly that of the mediastinum. When the fibrin was stripped off the pleura, the underlying membrane was shiny and showed no injection of the vessels. This was true of the whole pleura excluding that immediately covering the pericardium, where it was usually inflamed and somewhat shaggy. There were, in fact, no signs of pleural inflammation, except in that part which lay directly over the pericardium. The lungs were collapsed, and except in the very rarest cases showed no trace of œdema.

The pericardium was usually a little thickened, but in no case was there any evidence that it was either dense or hard enough to constrict the heart or interfere with its action. Pericardial effusion was never seen. In no instance was there evidence of obstruction to the great veins, either systemic or pulmonary, as they pierced the pericardium to enter the heart. The heart itself was sometimes normal and sometimes abnormally flabby. In the cases in which gelatine and calcium chloride were injected the flabbiness was extreme and, after removal, the mushroom-like collapsing of the heart left no doubt that a toxic myocarditis was present. Microscopy of the myocardium in cases treated with Dakin's fluid and tincture of iodine showed no definite changes; there was certainly no fatty degeneration. In many cases, however, a change was seen in the nuclei, which had become fragmented and appeared to lie in vacuoles in the cytoplasm. But this change may well have been an artefact, and it was felt that little weight could be attached to it.

The mediastinal tissues were œdematous, and much serum oozed from them when they were opened. A characteristic finding was that the mediastinal glands were greatly enlarged, turgid with fluid, and of a pale translucent plum-colour. Microscopy showed the sinuses widely distended with coagulated lymph containing large numbers of red cells

and lymphocytes. Innumerable dilated tortuous lymphatics were seen leading to and from these glands. The lymphatics were readily visible with the naked eye and often attained the size of the normal thoracic duct.

Frequently, but again not always, there was an effusion into the peritoneal cavity. The liver was always congested and nutmeg in appearance, and sometimes local areas of yellow necrosis were seen. Under the microscope venous congestion was not always seen, but a wide distension of the sinusoids with œdema fluid was an invariable finding. The intestines were always empty and distended with quantities of gas. The mesenteric and systemic veins were congested.

This post-mortem picture was built up on the appearances found in some 80 rabbits in which injection of one or other of the substances mentioned above had been made into the pericardium.

In order to emphasise the identity of the post-mortem appearances a summary of two protocols is appended.

Rabbit 0635. Weight 2020 g.

17/5/39. Operation: Pericardium approached by the usual route. 0·3 c.c. tinct. iodi mitis injected into pericardium. No immediate effect on the heart was apparent.

20/5/39. Well. Radiographed. No effusions.

24/5/39. Well. Radiographed. No effusions.

26/5/39. Dyspnœic. Œdematous. Radiographed. Bilateral effusions. Chest tapped. 25 c.c. removed from right chest; 20 c.c. from left chest. Given 8 c.c. urethane and killed after acute experiment.

Post-mortem.—Gross œdema of neck, chest wall, scrotum, and abdominal wall. Bilateral pleural effusions. Patchy collapse of lungs. Pleura normal except over pericardium. Heart flabby and small. Trace of oily blood in pericardium. Pericardium slightly thickened. Liver congested and showed a red reticular pattern on the surface. Large, congested, plum-coloured mediastinal glands. Many dilated lymphatics in the thorax, especially a large plexus anterior to the aorta. No ascites. Gut congested and full of gas. Mesenteric and systemic veins engorged.

Rabbit 0691. Weight 2080 g.

21/6/39. Operation: Pericardium exposed by usual route. 0·5 c.c. full strength Dakin's fluid injected into pericardium. Immediate dilatation and slowing of the heart was seen.

29/6/39. Radiographed. Heart shadow obscured by large right pleural effusion. 5 c.c. of fine suspension of Hydrokollag 300 injected into the effusion.

30/6/39. Killed after acute experiment.

Post-mortem. Scrotum œdematous. Bilateral pleural effusions. The Hydrokollag was loculated in a fibrinous mass just above the

diaphragm. Much fibrinous cobweb in both pleural cavities. Pericardium lightly adherent to the heart but not thickened. Lungs collapsed. Enormous pale mediastinal glands distended with colourless fluid. Ascites 50 c.c. Nutmeg liver. Congested bowel full of gas.

These two experiments are representative of the fully developed condition found in rabbits treated with (*a*) tincture of iodine, and (*b*) Dakin's fluid, and they serve to show the identity of the post-mortem appearances in each case.

Investigations made on the Preparation.

I. *To test the Hypothesis of Increased Capillary Permeability and Lymphatic Obstruction.*

Dible and Lynch postulated an increase in the permeability of the pleural capillaries. They reached this conclusion because they found that the effusions were stained blue after the intravenous injection of Trypan Blue. They stated that the dye must have escaped from the capillaries into the effusion, and alleged that this was evidence of abnormal capillary permeability. Although I have never used Trypan Blue, several experiments were made for other purposes in which Pontamine Blue 6 BX was injected intravenously into normal rabbits a short while before they were killed. In all cases where peritoneal fluid was present in the abdomen—there are often a few c.c. of fluid in the normal rabbit's abdomen—this was well stained by the dye. It would appear, therefore, that the rabbit's capillaries are normally permeable to dyes, and that the normal body fluids will become stained after intravenous injection. The staining of the effusions cannot be accepted as evidence of unusual capillary permeability.

Dible and Lynch also postulated an obstruction to the egress of fluid along the lymphatics between the pleura and the mediastinal glands. No mechanical obstruction was ever demonstrated, but they considered that the high protein content and viscosity of the exudate rendered it unable to traverse the lymphatics of the inflammatory zone. Their evidence was based upon the finding that Indian ink injected into the pleural effusions failed to reach the mediastinal glands in four days. Indian ink injected into the pleura of the normal animal was always found to have reached the mediastinal glands after this interval.

Their experiments are open to criticism owing to the extreme liability of Indian ink to flocculate and become unabsorbable when in contact with fluids with a high protein content. The experiment already quoted, Rabbit 0691, is a clear indication of this. Even the Hydrokollag 300, which is much less liable to flocculate than Indian ink, had become inspissated, and after 24 hours was loculated in a position which rendered its absorption impossible. Moreover, the distension of the lymphatics and the size of the mediastinal glands strongly suggest

that the lymphatics were doing more, rather than less, than their normal share of work. The following experiment appears conclusive:—

Rabbit 0570.

22/3/39. Pericardium approached by usual route. 0·25 c.c. tincture iodi mitis injected into pericardium.

30/3/39. Animal killed after acute experiment under urethane.

Post-mortem. Showed bilateral pleural effusions—20 c.c. each side. Very slight œdema. Congested liver.

Acute Experiment.—The thoracic duct in the neck was cannulated indirectly through the jugular vein after tying off all the tributaries and the superior vena cava. The cannula then lay in a closed venous sac into which the only entry was the thoracic duct. Heparin was injected intravenously to prevent clotting. A good flow of slightly blood-stained lymph was obtained from the cannula. 10 c.c. of slightly blood-stained fluid were then withdrawn from the left pleural cavity, and 5 c.c. of 5 per cent. Pontamine Blue 6 BX in normal saline were injected into the effusion in its place. Twenty minutes later the lymph escaping from the thoracic duct was lightly but clearly stained with blue. Blood was then withdrawn from a vein, and the animal killed. After centrifuging this blood the serum showed no trace of blue.

This experiment shows clearly that dyes injected into the pleural effusion are recoverable from the lymph in a short space of time. The dye could not have reached the lymph by way of the blood-stream, because at this time the serum itself was unstained. The only explanation is that the effusion and its contained dye were being rapidly absorbed through the lymphatics. It would appear, therefore, that the path of lymphatic absorption is intact. In view of the dilatation of the lymphatics and the distension of the glands, it is probable that the lymphatics are carrying along their path a larger than normal volume of fluid.

II. *Observations suggesting that Failure of the Heart is the Causal Mechanism.*

(a) *Morbid Anatomy.*—The morbid anatomical findings, when considered in their entirety, are highly suggestive of heart failure. The constancy and predominant nature of the pleural effusions are apt to lead one astray if due weight is not given to the other findings. This is probably the reason why Dible and Lynch turned to other factors for an explanation.

In addition to pleural effusions, all the animals showed congestion of the liver; many showed cutaneous œdema, and quite a large proportion showed ascites as well. These findings in themselves should suggest cardiac failure.

At the same time as these experiments were being made, Dr. A. N. Drury was working on the effects of arteriovenous aneurysms produced experimentally in rabbits. The method used was to divide one internal carotid artery and to unite the proximal end with the jugular vein by side to side anastomosis. He found that a small percentage of animals so treated developed anasarca and died suddenly. Post-mortem showed large bilateral pleural effusions and a picture in the rest of the body identical with that found in the animals into whose pericardia Dakin's fluid or iodine had been injected. This correspondence extended even to the dilatation of the thoracic lymph channels and to the enlargement of the mediastinal lymphatic glands. There was great enlargement of the heart, engorgement of the systemic veins and liver, cutaneous œdema, and sometimes ascites.

Protocol.

(1) Anastomosis, 2nd February 1939. March 1: Ascites suspected. March 8: Scrotal œdema obvious and gross ascites. March 9, 1939: Animal killed, 500 c.c. of ascitic fluid withdrawn. Scrotal œdema very definite. Pleural effusion. bilateral, small in amount. Thoracic lymph channels slightly dilated. Mediastinal lymphatic gland slightly enlarged. Liver engorged. Generalised œdema indefinite.

(2) Anastomosis, 2nd February 1939. March 10: Slight generalised œdema. Killed 17th March. Bilateral pleural effusion, blood-stained and considerable in amount. Thoracic lymph channels dilated. Mediastinal lymphatic gland enlarged. Ascites moderate in amount. Liver ungorged. Gross generalised œdema.

In these cases there had not been the slightest interference with the thoracic viscera, the pleural capillaries were undamaged, and the mediastinal tissues were not the seat of experimental inflammation. They show clearly that a condition which could be none other than a failure of the heart, occasioned by a large arteriovenous shunt, could produce post-mortem appearances identical with those found in the animals under discussion.

(b) *Venous Pressures.*—While the post-mortem evidence is suggestive, the results of experiments on the circulatory dynamics of these animals are quite convincing.

In taking venous pressures the method used was to pass a long glass cannula down the jugular vein to the base of the heart. The cannula was filled with citrate solution and was connected to a manometer. In all cases the thoracic walls were intact. Readings from the manometer were taken in every case in reference to a zero line passing horizontally through the base of the heart. This zero line was measured directly in each case after the death of the animal, the thoracic walls having been removed and the heart completely exposed.

Readings of the venous pressure at the base of the heart were taken

in 28 animals as normal controls. Their range, in centimetres of water, will be seen from fig. 1. The readings were nearly always at or below zero. While readings were occasionally as low as -3 or even -5 cm. water, the highest normal reading was $+2{\cdot}2$; this reading occurred only once and was never exceeded.

In the normal animal it was noticeable that the venous pressure fluctuated with each respiration, and a distinct movement of the column

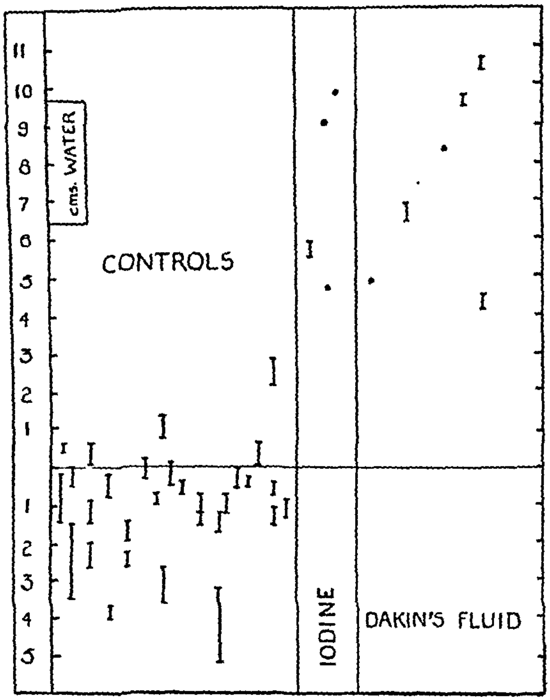

FIG. 1.—Venous pressures taken at the base of the heart. 1. Controls; 2. After injection of iodine into pericardium; 3. After injection of Dakin's fluid into pericardium.

of blood could be seen in the cannula where it met the citrate solution. In the pathological animal it was noticeable that this respiratory fluctuation was either diminished or obliterated. This is graphically represented in fig. 1, where two points joined by a line represent the variations in pressure in each animal. It will be noticed that in the pathological animals these pressure fluctuations have been damped out, and in some entirely obliterated. In these animals fluttering pulsations of the veins coinciding with the heart-beat were seen. Sometimes the veins pulsated almost like arteries, and it seems likely that in these animals an incompetence of the tricuspid valve was present, the result of dilatation of the a–v ring.

Ten observations on the venous pressure were made in the patho-

logical animals during acute experiments after which the animals were killed. All had pleural effusions. It will be seen from fig. 1 that the venous pressure was raised to a gross extent in every case, and that the rise was as significant in those treated with iodine as in those treated with Dakin's fluid. Whereas the normal venous pressure in the rabbit is in the neighbourhood of $\pm$ 1 cm. water, in those treated with iodine it varied from $+4{\cdot}8$ to $+10$ cm. water and in those treated with Dakin's fluid it varied from $+4{\cdot}5$ to $+10{\cdot}8$ cm. water.

(c) *Enlargement of the Heart.*—It has already been stated that direct visual observation at the time of operation shows that the heart dilates immediately the irritant is injected into the pericardium. This acute dilatation is greater when Dakin's fluid is injected than after the use of iodine.

It was decided, therefore, to ascertain whether there was any permanent increase in the size of the heart. For this purpose the animal was radiographed before operation in order to obtain a control tracing for each individual. Following operation it was radiographed again, and in a few animals a series of plates were taken on successive days. The outline of the heart was then traced from the film on to a strip of transparent cellophane, and finally transferred to paper. The results are seen in fig. 2.

In these figures the outline of the heart after operation has been superimposed on its outline before operation in order to show the difference in size. No attempt has been made at accurate superimposition, so that the tracings only give information about general enlargement of the heart. They give no indication of the chamber of the heart which was enlarged. It must be emphasised, however, that the method of radiography employed ensured that the animal was in the same position when each photograph was taken. It is certain, therefore, that each tracing provides accurate information about the absolute size of the heart at any given time.

It will be seen from fig. 2 that in nearly every case a significant increase in the size of the heart was demonstrable after operation—indeed it was seen at the operation itself—and it persisted until the death of the animal. As pericardial effusions were absent at post-mortem it is clear that the increase in the cardiac shadow was due to enlargement of the heart itself.

Increase in the size of the heart may be caused by two conditions, namely, hypertrophy and dilatation. As it is clear that there was insufficient time for hypertrophy to develop, there is no doubt that in these animals the increase in the size of the heart was caused by dilatation.

It may perhaps be argued that the rise in venous pressure was not the cause of the pleural effusions but rather resulted from them; that the increase in intrathoracic pressure due to the effusion was the

immediate cause of the rise in venous pressure. Unfortunately, no readings of the venous pressure at the base of the heart could be taken

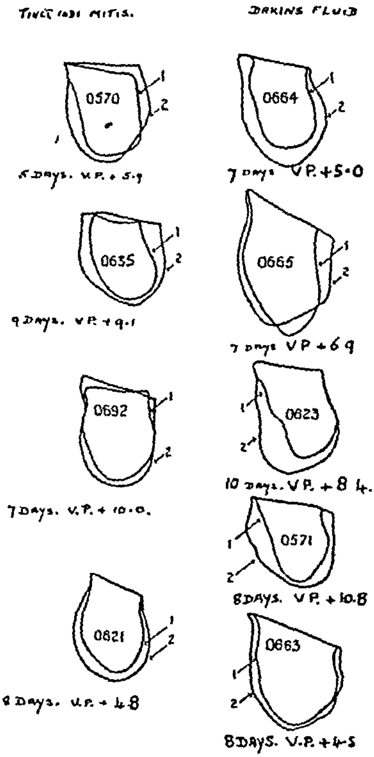

FIG. 2.—Tracings of rabbit hearts taken before and after operation. 1. Tracing from the normal animal before operation; 2. Tracing taken after operation. In each case the number of days after operation is indicated, followed by venous pressure value in cm. of water. The venous pressure recorded at the time of the final acute experiment is given in each case and is expressed in centimetres of water. The same venous pressures are recorded graphically in fig. 1.

before the onset of the effusions without killing the animal, and there is, therefore, no information as to the time of appearance of these two

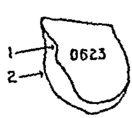

FIG. 3.—Rabbit 0623. 1. Tracing of the heart before operation.

16/5/39. 0·5 c.c. Dakin's fluid injected into pericardium.
20/5/39. Radiographed. Tracing No. 2 made from film. Radiography at this time showed no effusion.
24/5/39. Radiographed. Heart still enlarged. Small right-sided pleural effusion.

factors. On the other hand, there is no doubt that the dilatation of the heart preceded the onset of the effusions. Fig. 3 shows that in the case of Rabbit 0623 there was marked dilatation of the heart four days

after operation, but that signs of effusion did not appear until a further four days had elapsed. It is contended, therefore, that the combination of cardiac dilatation and a rise of venous pressure, when taken in conjunction with the post-mortem findings, offers unquestionable evidence that the condition of these animals is occasioned by failure of the heart. The absence of significant histological changes in the heart muscle which caused Dible and Lynch to discard the hypothesis of cardiac failure is undisputed, but it is felt that the present evidence from the living animal far outweighs this consideration and demonstrates clearly that cardiac failure is indeed the cause of the syndrome.

III. *Observations made on the Preparation.*

The foregoing remarks will have made it clear that a condition closely resembling congestive cardiac failure in man can be readily induced by experimental procedures in the rabbit. The preparation offers an excellent opportunity for the study of the dynamics of cardiac failure, and a number of investigations had been planned and would have been carried out had it not been for the outbreak of war. The few observations made, however, are perhaps worthy of record.

(1) *Diminution of the Pressure Gradient in the Great Veins.*—The pressure in the jugular vein of the rabbit may easily be taken by inserting the cannula into a large branch which joins the vein some distance below the angle of the jaw. If the point of the cannula lies in the branch just above its junction with the main vessel, the pressure reading taken will represent the lateral pressure in the main trunk itself. From this position the cannula can be rapidly slipped down the lumen of the jugular vein to the base of the heart, and a reading can then be taken in this situation. In the normal animal there is a well-marked pressure gradient between the jugular vein and the entry of the great veins into the heart itself. In the few normal animals that were studied the difference of pressure between these two situations was 5·8 cm. water. Five observations were recorded on rabbits in which an experimental failure of the heart had been induced (Table I). In all cases there was a very marked diminution in the pressure gradient between the jugular vein and the base of the heart; in three animals the gradient had been so decreased that it was impossible to measure it. The pressures were high in both situations, but the difference between them had been greatly diminished.

This observation is of importance as it helps to explain the nature of the failure of the circulation on the venous side of the heart. It is obvious that the venous return depends not only upon the hydrostatic pressure in the veins, but also upon the pressure gradient which exists along the course of the venous tree. No matter how high the venous pressure may be, no blood will flow towards the heart unless the pressure

in the peripheral veins is higher than that in the great venous trunks. The velocity of blood-flow in a vessel varies directly with the pressure gradient which occurs along its course, and any diminution of this gradient will occasion also a decrease in the velocity of blood-flow. It follows, therefore, that in these animals suffering from heart failure there must have been a greatly diminished rate of inflow of blood into the heart in spite of the rise in venous pressure.

Table I.—Venous Pressures in Centimetres of Water.

Normal animals.			Animals in heart failure.			
Jugular vein.	Base of heart.	Difference.	No.	Jugular vein.	Base of heart.	Difference.
6·9	−1·3 to −0·9	8·0	0692	+12	+10·0	2·0
8·2	+2·2 to +2·7	5·8	0691	+ 9·6	+ 9·6	0
			0663	+ 4·3	+ 4·3	0
6·3	+0·3 to +0·8	5·8	0665	+ 7·0	+ 6·5	0·5
6·0	+0·3 to +0·8	5·5	0664	+ 8·0	+ 5·0	3·0

(2) *Maintenance of the Arterial Pressure while the Heart is Failing.*—It is well known in clinical medicine that a failure of the heart does not always occasion a fall in systemic arterial blood-pressure. The preparation under discussion conforms to this finding, for in all of six animals which were investigated from this standpoint no significant fall in arterial pressure occurred during the period of cardiac failure.

The method adopted for measuring the arterial blood-pressure without destroying the animal has already been briefly described. It provides a figure for the systolic blood-pressure only. It is, of course, not a method of great accuracy, but the experimental error is probably not greater than ± 10 mm. Hg. In all cases a minimum of four readings was made and the mean of these taken as the final reading. Readings were usually taken on four successive days before operation in order to establish a control for the animal under observation. Readings were then taken at varying intervals after operation. At the final acute experiment the systolic and diastolic pressures were taken by the direct method after cannulating the carotid.

Representative results of two experiments are plotted in fig. 4 and show that up to and including the day of the final acute experiment, when the animals were proved to be in failure, no significant fall in arterial pressure had taken place.

It would seem, therefore, that in the heart failure manifested by these animals there was a redistribution of blood to the venous side.

This was reflected in the rise of venous pressure. The velocity of the blood in the veins was also slowed, and its inflow into the heart was impeded. These deductions follow from the reduction in the venous-pressure gradient. Yet the systemic arterial pressure remained at its normal level, *suggesting that the left ventricle was doing its work with unimpaired efficiency.* The absence of either œdema or congestion of the lungs supports this hypothesis. It is suggested, therefore, that in these animals there is a failure of the heart which is much more marked on the right than on the left side. It is, in effect, a preponderating right-sided heart failure.

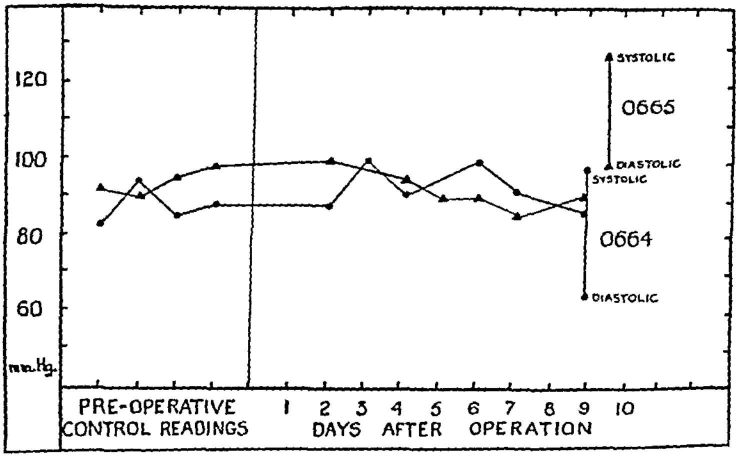

FIG. 4.—Arterial pressures before and after operation.

It is possible that the irritant injected into the pericardium produced a toxic myocardial degeneration of the superficial fibres of the heart. If this is true, the effect would be felt over a definite depth of cardiac muscle all over the surface of the heart. As the right ventricle is much thinner than the left, a relatively greater proportion of its thickness would be damaged by the irritant, and a correspondingly greater degree of failure of the right ventricle would be manifested.

(3) *Observations on the Protein Content of the Effusions.*—It was thought at first that information might be obtained either for or against heart failure as a cause of the syndrome from a study of the protein content of the effusions. If the effusions were inflammatory in nature, as suggested by Dible and Lynch, it was thought likely that they would show the characters of an exudate, with a high total protein content and a relatively low albumen-globulin ratio. If, on the other hand, heart failure was the cause, it was thought that they would prove to be transudates with a low protein content. All estimations were done by the micro-Kjeldahl method.

As has been shown elsewhere, there is no doubt that the cause of the

condition was a state of failure of the heart. There is, therefore, no doubt that the effusions were in fact formed by a process of "transudation." As will be seen from a study of Table II., the protein content

TABLE II.—SHOWING THE PROTEIN CONCENTRATIONS OF SERUM AND ASCITIC AND PLEURAL EFFUSIONS IN RABBITS SUFFERING FROM EXPERIMENTAL HEART FAILURE. MICRO-KJELDAHL METHOD. MILLIGRAMS PER 100 C.C.

			Total protein.	Albumen.	Globulin.	A./G.
0360	Dakin's fluid	Pleural	3·92	3·32	0·60	5·55
		Ascitic	4·69	3·63	1·06	3·52
0361	Dakin's fluid	Serum	5·61	4·34	1·27	3·4
		Ascitic	4·13	3·32	0·81	4·09
0373	Dakin's fluid	Pleural	3·10	2·51	0·59	4·25
		Ascitic	3·70	3·08	0·62	4·95
0570	Iodine	Serum	5·52	4·24	1·28	3·3
		Pleural	3·77	3·12	0·65	4·8
0571	Dakin's fluid	Serum	3·67	2·60	1·07	2·4
		Pleural	2·45	1·75	0·70	2·5
		Ascitic	2·87	2·11	0·76	2·8
0623	Dakin's fluid	Serum	5·48	3·99	1·49	2·7
		Pleural	4·13	3·04	1·09	2·8
0635	Iodine	Serum	5·52	4·44	1·08	4·1
		Pleural	3·01	2·24	0·77	2·9
0379	Gelatine calcium chloride solution	Pleural	3·09	2·64	0·45	5·9
		Ascitic	3·89	3·27	0·62	5·2

of the effusions, both peritoneal and pleural, if taken alone, would have led one to believe that they were inflammatory in nature and caused by a process of "exudation." The total proteins were high, and in those cases in which simultaneous observations were made on the serum the effusion proteins and the albumen-globulin ratio often approached the figures for the serum itself. Moreover, fibrinogen was always present, and, to judge by the amount of clot, was often found in quite large quantities; it was never directly estimated. This again is no bar to the diagnosis of transudation, for fibrin is present even in the fluid normally found in the rabbits' peritoneum. Several estimations were made by the same method on this normal fluid, and the results are tabled in Table III. It will be seen that, in comparison with the pathological effusions, it is much less rich in protein. Nevertheless, its protein content is higher than might have been expected,

and at least reaches the figure which would normally be considered a transudate.

Table III.—Showing the Concentration of Proteins in the Normal Peritoneal Fluid of Rabbits. Micro-Kjeldahl Method. Milligrams per 100 c.c.

No.	Total Protein.	Albumen.	Globulin.	A./G.
0284	1·8	1·31	0·49	2·6
0298	1·38	1·41	0·23	4·9
0302	0·96	0·66	0·30	2·2
0281	1·65	1·32	0·33	3·9
0294	1·68	1·23	0·45	2·7

The logical conclusion to draw from these figures is that no hard and fast line, such as is usually accepted, can be drawn between exudates and transudates. The value of protein estimations in differential diagnosis is of less value than is generally believed.

This view is not out of keeping with the general teaching of physiology and pathology, for in reality both are produced by the same physical principles. Assuming a constancy in the value of the plasma proteins, the factors at work in producing effusion are the capillary pressure and the capillary permeability. In heart failure and in inflammation these are both increased. In inflammation the capillary pressure rises because of vascular dilatation, and the permeability is increased by the action of bacterial toxins; in heart failure the capillary pressure rises because of the rise in venous pressure, and the permeability is increased by anoxæmia resulting from reduction in the velocity of the blood. On theoretical grounds there would seem no reason to doubt that a severe rise in the venous pressure and a severe slowing of the circulation can produce the same mechanical factors as inflammation itself. While it cannot be denied that some effusions in their protein content are characteristic of the process of transudation, and others, of inflammatory nature, are typical of exudation, it must not be forgotten that the one type merges imperceptibly into the other. The overlap is of such dimensions that in all these animals differential diagnosis from the effusion itself was quite impossible.

Summary and Conclusions.

1. The effect of injecting Dakin's fluid and iodine into the pericardium of the rabbit is described.

2. The resulting changes are shown to be caused by heart failure, and not by inflammation and lymphatic obstruction as heretofore believed.

3. The preparation is a simple method of producing experimental failure of the heart.

4. In these animals, in addition to a rise of venous pressure, a reduction of the pressure gradient in the veins was demonstrated.

5. During the course of the heart failure the arterial pressure remained constant.

6. From investigation of the protein content of the effusions the conclusion was drawn that too rigid an interpretation of the characters of transudates and exudates cannot be accepted. In the type of heart failure under discussion the effusions have the characteristics normally ascribed to exudates.

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THE MECHANISM OF CAPILLARY CONTRACTION. By A. G. SANDERS, R. H. EBERT, and H. W. FLOREY. From the Sir William Dunn School of Pathology, Oxford.

(*Received for publication 9th July* 1940.)

KROGH [1929] in his well-known book on the capillaries summarised the literature up to that date on the question of capillary contraction and the mechanism by which it was produced. The views which he there expressed are now almost universally accepted by authors of text-books. Briefly he considered the evidence was sufficient to show that the independent contractility of capillaries was due to the presence on their walls of adventitial cells—Rouget cells—which contracted, and by means of protoplasmic processes surrounding the capillary caused the narrowing of the lumen.

Since that summary appeared further work has been done which shows that the position is by no means so clear-cut.

Some investigators [Kahn and Pollack, 1931; Field, 1935; Beecher, 1936 *a* and *b*] described active capillary contractions, but others [Sandison, 1931; Clark and Clark, 1935; Zweifach, 1939] concluded that no active contraction occurred and that capillaries played only a passive part in the control of the circulation. The Clarks [1935] state that although they saw active contraction of capillary endothelium in amphibian larvæ, they could find no true contraction of capillaries in the rabbit's ear. Sandison [1931] described changes in the calibre of capillaries as passive, depending chiefly on pressure changes inside (and outside) the capillary, coupled with a certain elasticity of the endothelium. Zweifach [1934] has observed contraction of capillary endothelium in response to direct stimulation, but thinks that normally "the changes in capillary diameter which do occur are slow and passive" [Zweifach, 1939]. He states that diminished intracapillary pressure and good endothelial tone cause a gradual narrowing of the capillary lumen over a period of 15 to 30 minutes. He believes much confusion arises from the fact that there is no adequate definition of the term "capillary." He points out that many observers have probably confused "arteriovenous bridges," which possess a few scattered muscle cells and contract actively, with true capillaries, which have no muscles and contract passively.

Beecher [1936 *a*, *b*] made observations on a "preformed tissue"

chamber in the ear of a rabbit, and did not verify the Clarks' findings. He described active contractions of capillaries in the rabbit after various stimuli. The "preformed tissue" type of rabbit-ear chamber contains vessels having their normal innervation, but suffers from the disadvantage that the thickness of the tissue is so great that accurate observations with high powers of the microscope cannot be made.

The Mechanism of Capillary Contraction.

Two mechanisms have been postulated to explain capillary contractility: (*a*) Contractile perivascular elements (Rouget cells), and (*b*) endothelial contraction.

(a) *Rouget Cells.*—Krogh [1929] summarises the evidence for this. Later observers agree that capillary contractions are accompanied by longitudinal folds in the wall of the capillary [Kahn and Pollack, 1931], and that there is an actual decrease in the external diameter of the capillary [Field, 1935]. Bensley and Vimtrup [1928] studied supravital preparations of the nictitating membrane, and described myofibrils in Rouget cells which stained specifically with Janus green B.

The theory that Rouget cells are active contractile elements has been criticised by Sandison [1931], Clark and Clark [1935], and Zweifach [1936–37]. Using rabbit-ear chambers, Sandison and the Clarks found no evidence that Rouget cells play a part in capillary contraction. They claimed that capillaries sometimes contracted (passively) away from adventitial cells. Zweifach [1934] made observations on capillaries in the tongue, nictitating membrane, mesentery, and intestinal wall of the frog, and actually stimulated individual adventitial cells with micro-needles. Although a Rouget cell rounded up in response to the stimulus, there was no contraction of the capillary. He says that longitudinal folding does not necessarily denote the presence of a contractile adventitial cell since an inert cell which simply adheres to the capillary wall could cause folding [Zweifach, 1936–37]. He also repeated the Janus green experiments of Bensley and Vimtrup [1928], and concluded that the dye stained connective-tissue fibres in the region of the capillary and not myofibrils.

(b) *Endothelial Cells.*—Having failed to find positive evidence for the contractile nature of Rouget cells, Clark and Clark [1925], Florey and Carleton [1926], and Zweifach [1934] suggested endothelium as the active factor. Zweifach states "Mechanical stimulation of the endothelium, especially at the nuclear nodes, causes a localised constriction of the capillary wall with the appearance of ridges or folds in the surface, and accompanied by a thickening and bulging of the endothelial nucleus into the lumen of the vessel."

Swelling of the endothelial nucleus into the lumen of a constricted vessel has also been described by Kahn and Pollack [1931] in the

nictitating membrane, Field [1935] in the mesentery of the rat, and Beecher [1936 *a*) in the ear of the rabbit.

Kahn and Pollack [1931], Field [1935], and Beecher [1936] have combined the two divergent views and describe two types of capillary contraction: (*a*) Contraction of Rouget cells accompanied by longitudinal foldings and a decrease in the external diameter of the capillary, and (*b*) contraction caused by swelling of endothelial nuclei in which there is little change in the external diameter of the vessel.

The observations to be described in this paper were made, in the first instance, incidentally, during the course of experiments with a modified Sandison-Clark chamber (Ebert, Florey, and Pullinger, 1939) in which the behaviour of tissue cells was being watched. In this type of chamber newly grown tissue of extreme thinness—30 μ or less—can be obtained. It is possible to see the small vessels with greater clarity and more highly magnified than by any other means at present available. For visual observation a 2-mm. Leitz apochromatic objective in conjunction with 7 × oculars in a binocular body (Leitz) was used. The photographs were taken on Kodak E.F. panchromatic film with a Leitz Mikas attachment using the same objective and a 10 × periplanatic eyepiece.

The spontaneous contraction and dilatation which appeared in the finest capillary vessels can perhaps be best appreciated by reference to the photographs (figs. 1, 2, 3, 4, and 5).

Many observations were made in a number of chambers, but the contractions were never visible in all capillaries—what determined the condition of contractility was not apparent.

Observations of this kind left us unable to determine whether the adventitial cell really played a part in contraction. Contractions certainly took place in their neighbourhood, though no change in their shape could be determined with certainty. Swellings of endothelium which diminished the lumen were certainly observed.

In order to clarify this position further it was necessary to produce contractions at will. This was most simply and successfully accomplished by stimulation of the peripheral end of the cut cervical sympathetic nerves. Three rabbits carrying good chambers were used. The chambers had been in their ears for several months so that nerves had had ample time in which to grow on to the vessels.

The animal was anæsthetised with Nembutal (0·5 c.c. per kilo) given intravenously, supplemented by ether during the dissection of the cervical sympathetic nerve.

The nerve was prepared, but not tied or cut, and the chamber fixed to the stage in the usual way, the heating apparatus being put in place so that the ear should be about 37° C. (for details of this arrangement, see Ebert, Sanders, and Florey, 1940). A suitable capillary was then selected and watched with the oil-immersion lens. Firstly, the intact

sympathetic was faradically stimulated (30 make-and-break shocks per second), which produced contractions. The sympathetic was then cut. This greatly increased the speed of flow through the vessels of the ear-chamber, with dilatation of the arterioles.

Before stimulation of the peripheral end of the sympathetic the effect of pinching the common carotid was observed. This greatly reduced or stopped the flow through the ear, and it is certain that the intravascular pressure must have been quite small. Nevertheless, except in one instance, only the slightest diminution of the diameter of the capillaries was observed. Nor were the arteriole diameters much affected. The effect of stimulation of the nerve was then tried. In all three rabbits the swelling of the endothelial cell in the region of the nucleus to block the capillary lumen was clearly seen. The latent period from the beginning of stimulus was about 15 to 20 seconds, and the capillary remained contracted up to 45 seconds after the cessation of the stimulus. The Rouget cells present were not observed to undergo any change nor did the outside diameter of the capillary sensibly diminish during contraction. These effects were photographed (figs. 6, 7, 8, and 9).

Both the celluloid chambers of Clark and his collaborators and ours made of the plastic substance, perspex, suffer from the defect that the full numerical aperture of the condenser cannot be used owing to the thickness of material over which the tissue to be observed grows.

A chamber of the following design enables full use to be made of both condenser and oil-immersion lens, so that it may be said that for ordinary illumination the optical arrangements have been made as good as at present seems possible.

The base of the chamber is made of sheet-silver 0·6 mm. thick, in the centre of which is a circular hole 8 mm. diameter. A disc of perspex 1·1 mm. thick fits this hole tightly and is flush with the under surface. Both faces of the perspex are highly polished and are flat. As the silver is too thin for the bolts to be screwed into it, these are fixed with nuts on both sides. The total diameter of the chamber is 40 mm. to allow for lateral movement of the chamber relative to the condenser (a Watson holoscopic), which, with its top lenses in position, is rather broad. The buffers are put on in the usual way. The design of the lid has been slightly modified so as to lessen the "carry" of the mica coverslip which, with such a large chamber, would be likely to buckle unduly (fig. 10).

Insertion of the Chamber.

In general, the insertion is done as previously described [Ebert, Florey, and Pullinger, 1939], but certain modifications have to be made. A particularly broad ear is necessary, and, even so, the central artery

FIG. 1.

FIG. 2.

FIG. 1.—Capillary showing Rouget cell.

FIG. 2.—Same capillary contracted. An endothelial nucleus has become visible and the Rouget cell appears more closely applied to the wall, which is somewhat indented. This appearance was rarely seen. The contraction in this capillary was observed to occur "naturally."

FIG. 3.

FIG. 4.

FIG. 5.

FIGS. 3, 4, AND 5.—Contraction in a capillary. Note swelling of endothelial nuclei.

In fig. 5 the junction of the capillary with another vessel is contracted and a red cell is being squeezed through.

The Rouget cells present do not seem to be actively involved.

sympathetic was faradically stimulated (30 make-and-break shocks per second), which produced contractions. The sympathetic was then cut. This greatly increased the speed of flow through the vessels of the ear-chamber, with dilatation of the arterioles.

Before stimulation of the peripheral end of the sympathetic the effect of pinching the common carotid was observed. This greatly reduced or stopped the flow through the ear, and it is certain that the intravascular pressure must have been quite small. Nevertheless, except in one instance, only the slightest diminution of the diameter of the capillaries was observed. Nor were the arteriole diameters much affected. The effect of stimulation of the nerve was then tried. In all three rabbits the swelling of the endothelial cell in the region of the nucleus to block the capillary lumen was clearly seen. The latent period from the beginning of stimulus was about 15 to 20 seconds, and the capillary remained contracted up to 45 seconds after the cessation of the stimulus. The Rouget cells present were not observed to undergo any change nor did the outside diameter of the capillary sensibly diminish during contraction. These effects were photographed (figs. 6, 7, 8, and 9).

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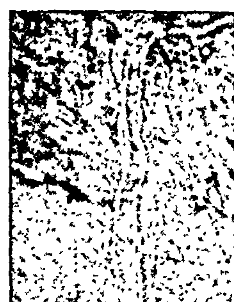

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SANDERS, EBERT, and FLOREY, "The Mechanism of Capillary Contraction"

FIG. 6.

FIG. 7.

FIG. 8.

FIG. 9.

FIGS. 6, 7, 8, AND 9.—Photographs taken during stimulation of the cervical sympathetic nerve.

Fig. 6.—Before stimulation. Note endothelial cell, E, and Rouget cell, R.

Fig. 7.—During stimulation. Note swelling of endothelial nucleus and no change in Rouget cell.

Fig. 8.—Further swelling of endothelial nucleus. The lumen of the vessel is now completely obliterated. Rouget cell shows no change, and there is no significant alteration in the external diameter of the capillary.

Fig. 9.—Dilatation again. One minute after cessation of stimulus.

runs across the position of the table. For this reason the central hole cannot be punched out first, but has to be cut out with scissors after the dissection. The punching of the three holes in the ear to take the bolts and the dissection of the skin flaps is done as usual. The cartilage is then dissected away over an area of 24 mm. in diameter. This is done from the inner side of the ear, the perichondrium carrying the

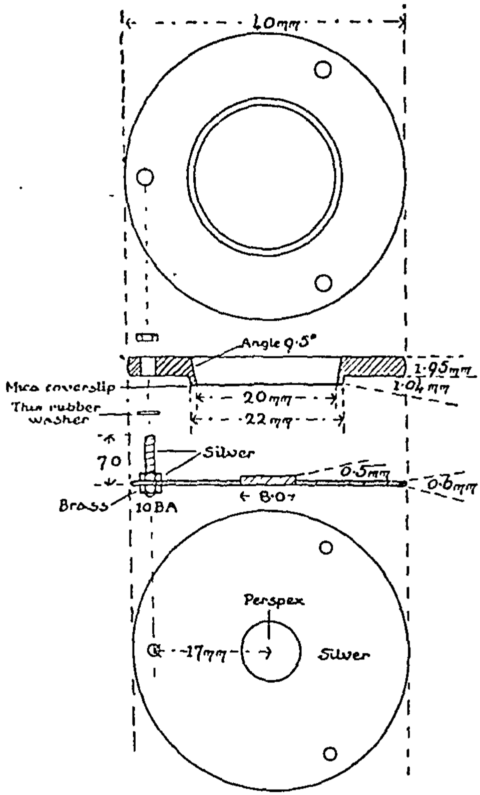

FIG. 10.—Diagram of chamber with optimal optical properties.

vessels on the outer side being left. A hole to fit the table is then cut in this remaining thin layer of tissue, the main artery being retracted to one side. The two parts of the chamber are screwed together as usual. In the course of some weeks the central table is vascularised as in other types of chambers.

A chamber of this type was used for experiment 83 days after its insertion and about 45 days after complete vascularisation. Optically the results were very clear. There was no question whatever that in many capillaries the endothelial nucleus appeared to swell and block the lumen, but no movement was observed in the Rouget cells after stimulation of the peripheral end of the cervical sympathetic nerve.

The latent period was short—about 5 to 7 seconds—and the contraction after a 5-second stimulus lasted about 15 to 30 seconds. This result was repeatedly obtained on many capillaries. A cinema film in colour was made, using a 1/16-inch oil-immersion objective with a 12 × eyepiece.

A phenomenon not previously described has often been noted in venules; *it is usually seen after mild trauma.* "Spikes" appear to form on the endothelial surface on which the red corpuscles of the blood-stream are caught momentarily, and are thereby grossly deformed. We have not been able to determine the nature of these "spikes" as they are themselves invisible and are only revealed by the deformation of the red cells caught on them (fig. 11). These "spikes" are often very numerous; they do not appear to have any relationship to platelets.

Summary.

(1) From this series of experiments carried out under good optical conditions, in one case as good as present lenses allow, we conclude that capillary contraction occurs in the rabbit's ear-chamber, that this contraction is under the control of the sympathetic nervous system, and that endothelial swelling causes the obliteration of the vessels. No positive evidence was obtained of the participation of Rouget cells in this contraction.

(2) "Spikes" on the endothelium of slightly injured veins have been noted. Red corpuscles hang on these, often for several seconds.

The chambers have been made in the laboratory by Mr. S. W. Bush, to whose skill we are greatly indebted.

Mr. J. Kent has had charge of the animals.

The drawing is by Mr. H. Axtell.

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FIG. 11.—"Endothelial spikes." Note red cells caught on spikes and bent double by force of stream.

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SUGAR, WATER, AND CREATININE EXCRETION IN NORMAL AND IN B-AVITAMINOTIC DOGS.

By N. Hamar. From the Institute of Physiology of the "Peter Pázmány" University, Budapest.

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Experiments have been carried out, and are described in this paper, in order to obtain information concerning the glucose-absorbing power of the tubular epithelium of the kidney in a state of B-avitaminosis.

The prevailing supposition is that the glucose-absorbing power of the tubular epithelium is decreased in B-avitaminosis. This supposition is, however, only inferred from very indirect experimental evidence. This is as follows: In B-avitaminosis the body-cells take up glucose at a slower rate (for details of literature see Burack and Cowgill [1932]). The retardation of glucose absorption by the tissues in B-avitaminosis —as observed by following the blood-sugar changes—signifies, of course, largely a retardation of the sugar uptake by the liver and muscles. It appears, however, that epithelial cells are also affected, since the mucous membrane of the intestinal tract also absorbs at a reduced rate, as was demonstrated by Westenbrink and Overbeek [1933] on the intestines of the rat. As to changes in the functions of the kidney-cells in B-avitaminosis, we found no direct evidence in the literature. But that these cells are affected by the lack of vitamin B_1 has been proved by Thompson's [1934] experiments. The lack of vitamin B_1 causes a specific damage to the respiration of the kidney-cells. The experiments of Jonata [1932] on phlorrhizinised rabbits are noteworthy in this connexion: sugar-loading in such rabbits is accompanied by a great glycosuria, but this is almost completely inhibited by the simultaneous addition of vitamin B. Jonata's conclusions from these experiments were as follows: The site of action of phlorrhizin is mainly in the kidney; if, therefore, vitamin B decreases phlorrhizin glycosuria, its action is chiefly due to a re-elevation of the renal threshold. A few years later Campanacci and Ferretti [1935] confirmed Jonata's results, and corroborated his hypothesis by showing that although the blood-sugar values remain slightly higher than the normal ones, the glycosuria almost disappears.

In testing the supposition that the absorbing power of the tubular epithelium is decreased in B-avitaminosis we have carried out the following experiments.

autoclaved for 1 hour at 120° C. The B-vitamin was extracted from casein as follows: The casein was boiled with 0·03 per cent. acetic acid for 15 minutes, filtered and washed with tap water. This procedure was repeated twice, after which the washed casein was dried in an oven at a temperature of 70° C. Autoclaved rice, the B-vitamin-free casein, and salt mixture were cooked in water to a thick mush. The oils were added after cooling. The animals were not at all averse to the diet.

Results.

Out of the 13 B-avitaminotic dogs observed by us, the B-avitaminosis manifested itself in the acute spastic form in 3 cases; in the rest the chronic-paralytic form was observed. We cannot offer any explanation for this difference. In the acute spastic cases the administration of vitamin B_1 ("betaxin"), either intramuscularly or intravenously, caused the symptoms to disappear. Such treatment, however, was entirely without effect in the chronic-paralytic animals. The results published in this paper deal with the chronic-paralytic animals only.

In the first type of experiment five animals were used. While on normal diet they were loaded three times. Four of these five dogs showed a development of B-avitaminosis in its chronic-paralytic form. Each dog was loaded once in the prodromal, and twice in the fully developed stage of B-avitaminosis. In the second type of experiment, on five other dogs, loadings on each were made as follows: ten in the normal, seven in the prodromal, and five in the severe stage of B-avitaminosis. In each case a period of rest, extending over three or four days, intervened before the experiment was repeated.

In fig. 1 the blood-sugar values are demonstrated. Here we see that the blood-sugar level decreases in time, in the first 10 minutes rapidly, later slowly. The rates of the disappearance of glucose from the blood show no difference in the normal and in the prodromal stage. In either case the following fractions of the injected glucose disappear in time: 87·3 per cent. during the first 10 minutes, 5·6 per cent. during the second 10 minutes, 4·4 per cent. during the third 10 minutes, 1·8 per cent. during the 30–45 minutes, and 0·8 per cent. during the 45–60 minutes after sugar-loading. When the B-avitaminosis is well developed, a retardation is noticeable in the rate of disappearance of glucose from the blood. During the first 10 minutes after sugar-loading 82 per cent., during the second 10 minutes 3·5 per cent., during the third 10 minutes 3·5 per cent., during the 30–45 minute-period 3·8 per cent., and during the 45–60 minute-period 4·7 per cent. of the injected glucose disappears. The retardation of the sugar-disappearance in the avitaminotic dogs as compared with the normal ones was as follows: 6 per cent. during the first 10 minutes, 37 per cent. during the

Method.

In the first series of experiments on dogs, after a 24-hours' hunger period, 1 g. of sugar/kg. body-weight was given to the animals. The sugar was injected, in 25 per cent. solution, into the vena saphena. At times of 0, 10, 20, 30, 45, 60 minutes from the beginning of the experiment blood was taken from the vein in the ear and its sugar-content was determined by the method of Fujita and Iwatake [1931]. During the experimental period the urine was collected by means of a catheter at intervals of 0–10, 10–20, 20–30, 30–45, 45–60 minutes and its glucose content determined polarimetrically.

In the second series of experiments essentially the same methods as above were employed, but simultaneously with the sugar-loadings and determinations the creatinine clearance of the dogs was determined as follows: In about 4 c.c. distilled water 30–40 mg. of creatinine/kg. body-weight was dissolved and this solution was injected subcutaneously. About 20–30 minutes later, after the creatinine concentration of the blood had reached a well determinable level, glucose was administered as already described. The blood was taken from the saphenous vein at times of 0, 7, 15, 26, 40, and 60 minutes from the administration of glucose, and urine was collected at intervals of 0–10, 10–20, 20–32, 32–50, and 50–80 minutes. Creatinine and sugar determinations were made in the blood as well as in the urine. By thus employing the Rehberg [1926] test in combination with blood- and urine-sugar determinations, we have calculated the rate of production of glomerular ultrafiltrate and its glucose content. Such calculations are, naturally, based on an assumption which necessarily must be an arbitrary one. It is supposed that the sugar and creatinine concentration of the glomerular ultrafiltrate is identical with that of the blood-plasma, and that creatinine is neither secreted nor reabsorbed in the tubules. The sugar concentration of the plasma was calculated from that of the blood by taking the hæmatocrit value as being 50 per cent.

Perineotomised female dogs of 8–15 kg. body-weight were used. After repeated control sugar-loadings the animals were put on a B-vitamin-free diet. The sugar-loadings were performed again in both the prodromal (anorexia, loss of weight, depression, inco-ordination, muscular weakness) and the fully developed stages of the B-avitaminosis.[1]

The B-vitamin-free diet consisted of polished, autoclaved rice (300 parts), B-vitamin-free casein (50 parts), flax oil, cod-liver oil (15 parts of each), and McCollum salt mixture (12 parts). The rice was

[1] We do not wish to specify our expression as regards the kind of B-avitaminosis, because the diets used by previous workers and by us were certainly deficient in many dietary factors, though they were and still are generally regarded as B_1-avitaminotic diets.

The difference between the glucose lost from the blood and that passed with the urine is the sugar taken up by the cells or decomposed in the blood. In normal dogs and in those in the prodromal stage of

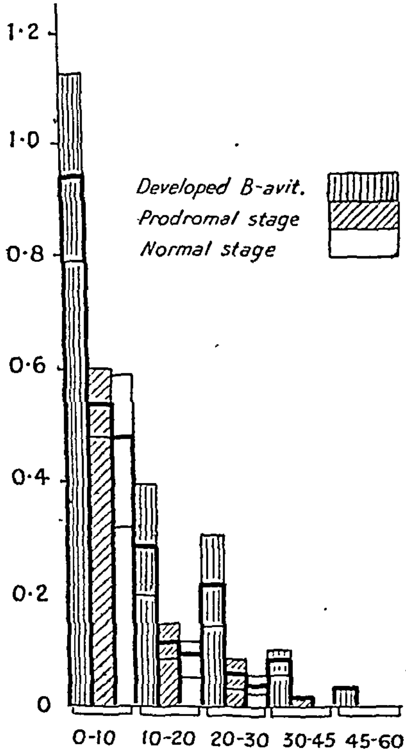

FIG. 2.—Curves for the sugar excreted in the urine during 10-minute period by 5 normal dogs and by 4 dogs in the prodromal and the fully developed stages of B-avitaminosis. Abscissa: time elapsed after sugar-loading in minutes as indicated in the figure. Ordinate: amount of glucose in the urine, g./10 kg. body-weight. The thin horizontal lines indicate scattering of the values round the averages, which are indicated by thick lines.

B-avitaminosis during the first 10 minutes, out of 8·73 g. glucose disappearing from the blood, 0·50 g. was excreted in the urine and 8·23 g. disappeared into the tissues. In the second period of 10 minutes, out of 0·56 g. glucose 0·10 g. was passed with the urine and 0·46 g. disappeared into the tissues. During the second 10 minutes the rate of the disappearance of glucose from the blood towards the tissues was decreased as compared to the first 10 minutes. From the beginning of the third 10-minute period the tissues took up 0·41 g. of glucose, whereas the glucose excreted in the urine was only 0·02 g. After the beginning of the second period of 10 minutes the rate of the sugar uptake by the tissues was therefore nearly constant till the blood-sugar level reached the renal threshold at the end of the third 10-minute period. The data obtained in fully developed B-avitaminosis are as

second 10 minutes, and 27 per cent. during the third 10 minutes. From the 30th minute onward the rate of disappearance increased, but the excess of glucose at the beginning of this period was so much

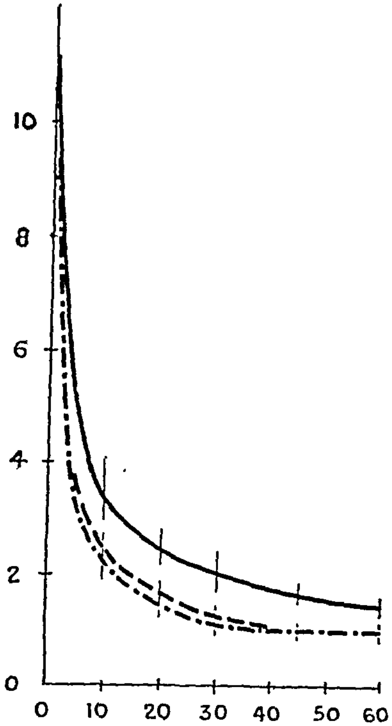

FIG. 1.—Blood-sugar curves of 5 normal dogs and of 4 of these during the prodromal and fully developed stages of B-avitaminosis. Abscissa: time in minutes after the intravenous injection of glucose. Ordinate: amount of glucose in the blood, g./10 kg. body-weight (in the calculation the blood-volume was taken as 8 per cent. of the body-weight). Solid line: dogs in fully developed avitaminosis. Broken line: dogs in the prodromal stage of avitaminosis. Dot-dash line: dogs in the normal stage. The vertical lines on the curves connect the maximum and minimum values obtained.

that at the end of the experimental period, in spite of the increase in the rate of sugar-disappearance during the second part, the amount of glucose in the blood (together with the 2·7 per cent. of injected glucose) was more than in the normal animals.

Fig. 2 shows the amounts of glucose excreted in the urine during the experimental period. Most glucose was passed during the first 10 minutes and later the excretion decreased by degrees. With normal dogs and with those in the prodromal stage of the B-avitaminosis the glycosuria existed practically in the first 30 minutes only. In the fully developed avitaminosis, glycosuria lasted till the end of the experiment, and the amount of sugar excreted during any given period was much larger in the avitaminotic dogs than in the normal ones.

experiment, at first rapidly and later slowly in close parallelism with the blood-sugar values as described in the first series of experiments. In the prodromal stage of B-avitaminosis the sugar concentration of the filtrate does not deviate from the normal. In the fully developed B-avitaminosis, however, the glucose concentration is higher than in the normal because of the higher blood-sugar level.

The behaviour of the total sugar curves is as follows: In normal dogs the total amount of glucose secreted in 10 minutes steadily and gradually decreases from the start of the experiment till its termination.

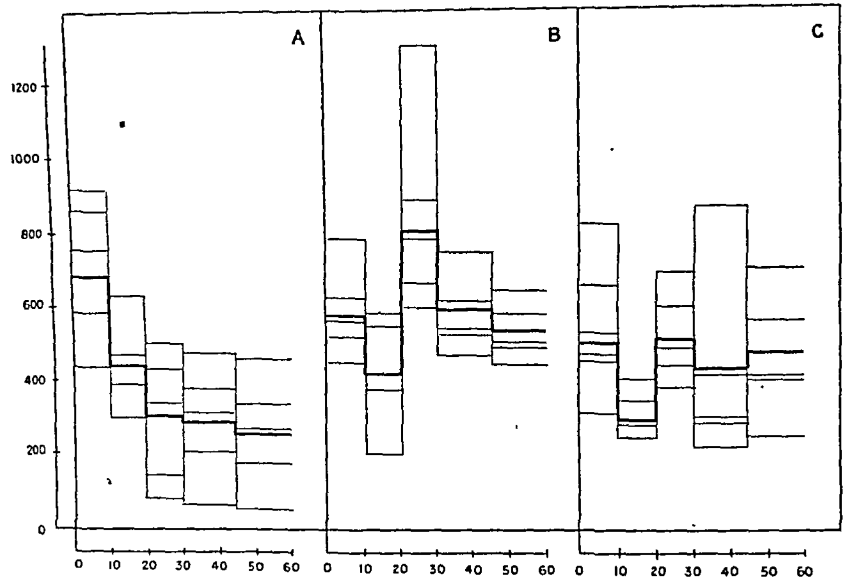

FIG. 4.—The volume of glomerular ultrafiltrates in c.c. during 10-minute periods. Abscissa: time in minutes after sugar-loading. Ordinate: the volume of the glomerular ultrafiltrate in c.c. Thin lines: scattering of values round the averages, which are indicated by thick lines. A: normal. B: prodromal. C: fully developed avitaminosis.

Whereas the decrease in the amount of sugar excreted in the urine by the normal animal is gradual, so that it may be represented by a hyperbolic curve, this is not so in the prodromal or fully developed stage of B-avitaminosis. In both of these there is a sharp but transient increase in the total amount of the sugar filtered. This increase lasts for only 12 minutes and in each case occurs at the same period of the time curve, *i.e.* during the 20–32-minute period.

This difference between normal and B-avitaminotic dogs is explained by the similar behaviour of the volume of glomerular ultrafiltrate in the corresponding phase of the time co-ordinate (fig. 4). In normal animals the volume of the ultrafiltrate falls as a rule gradually and smoothly from the start of the experiment till its end. In the prodromal and fully developed stages of B-avitaminosis this gradual and

follows: During the first 10 minutes, out of 8·2 g. glucose 0·95 g. was excreted in the urine and 7·25 g. were taken up by the tissues. The absorbing power shows therefore, already in the first 10 minutes, a slight decrease as compared with the normal. During the second 10 minutes the decrease in sugar absorption became more pronounced: out of 0·35 g. glucose 0·26 g. was excreted in the urine and only 0·09 g. was taken up by the tissues. In the third 10-minute period, out of 0·35 g. glucose 0·21 g. was returned in the urine and 0·14 g. was the share of the tissues. From the results of the periods 30–45 and of

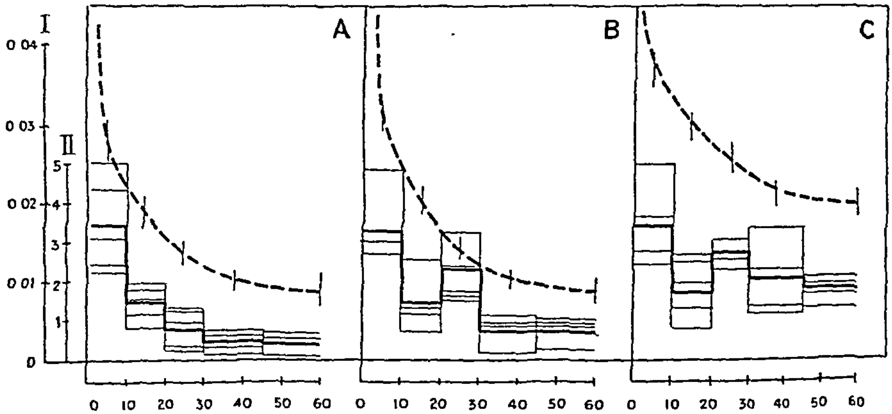

FIG. 3.—The concentration and the total amount of sugar filtered during 10-minute intervals in the glomeruli. Abscissa: time in minutes after sugar-loading. 1st ordinate: molar concentration of sugar in the glomerular filtrate; 2nd ordinate: total amount of sugar filtered during 10 minutes in the glomeruli. Broken lines: molar concentration of sugar in the glomerular filtrate. Solid lines: total amount of sugar (in g.) filtered during 10 minutes. Thin lines: scattering of the values round the averages, which are indicated by thick lines. A: normal. B: prodromal. C: fully developed avitaminosis.

45–60 minutes the rate of sugar disappearance/10 minutes may be calculated, and the figures thus obtained may be compared with those obtained during the preceding periods. The comparison clearly shows that during the 30–45-minute period the rate of sugar disappearance to the tissues was increased from 0·14 g./10 minutes to 0·21 g./10 minutes. During the period of 45–60 minutes the absorbing power of the tissues increased further from 0·21 g./10 minutes to 0·27 g./10 minutes.

In the second series of experiments the changes in the sugar-absorbing power of the tubular epithelium during B-avitaminosis have been studied. The sugar-absorbing power of the tubular epithelium at the different experimental periods was calculated from the following values: (1) blood-sugar concentration; (2) rate of glomerular ultrafiltration (creatinine clearance); (3) amount of glucose excreted in the urine.

Fig. 3 shows the concentration and the total quantities of sugar filtered in the glomeruli. The concentration curves show that the sugar concentration of the filtrate falls from the commencement of the

The quantity of sugar excreted during the different experimental periods is shown in fig. 5. A comparison of these results with those obtained in the first series of experiments (fig. 2) shows that the shape of the curve is the same in both cases. On the other hand, in this second type of experiment the quantity of glucose excreted is slightly larger in each period. This difference is no doubt due to the fact that in the meantime the B-avitaminosis became more severe.

A more interesting phenomenon is revealed by a comparison of figs. 5 and 3. This latter shows the variations in the quantity of glucose filtered and of its concentration in the glomerular filtrate during the different experimental periods. It is seen from fig. 5 that the amount of glucose excreted in the urine does not show an increase during the period of 20–32 minutes, as is the case with the amount of glucose in the glomerular filtrate, but it decreases steadily and smoothly from the beginning to the end, as does the concentration of glucose in the glomerular filtrate. This naturally means that during the period of 20–32 minutes the tubules absorb more glucose than they do during the preceding or following 10 minutes. In normal dogs the rate of absorption by the tubules was as follows: 3·10 g. during the first 10 minutes, 1·43 g. during the second 10 minutes, and 0·8 g. during the period of 20–32 minutes. After this period there was no glycosuria. In the prodromal stage of B-avitaminosis the same determinations gave values as follows: 2·69 g., 1·33 g., 2·06 g.; in the fully developed B-avitaminosis: 2·56 g., 0·93 g., 2·38 g. The rate of absorption by the tubules in the latter periods of the experiments on dogs suffering from B-avitaminosis was as follows: During the period of 32–50 minutes 1·76 g./10 minutes, and during the 50–80-minute period 1·67 g./10 minutes.

Discussion.

Regarding the mechanism of the glycosuria seen in B-avitaminotic dogs as a consequence of the decreased sugar tolerance, the following conclusions appear to be warranted. It is certain that in the production of the glycosuria the hyperglycæmia plays an important rôle. This hyperglycæmia is due—as is evident from our results—to a decreased disappearance of sugar from the blood. From the fact that in B-avitaminosis the body-cells take up sugar at a reduced rate, one is led to the supposition that the tubular cells of the kidney are not excepted from this general rule. If this is true, then it follows that in the production of the glycosuria decreased reabsorption of sugar by the tubular epithelium from the glomerular ultrafiltrate, as another component, also plays a rôle. A closer scrutiny of the experimental results, however, reveals certain interesting circumstances which must be analysed before the theory of a decreased tubular sugar reabsorption can be accepted.

even fall is absent. The volumes of the filtrate in the 20–32-minute period in comparison with the volumes of the previous 10-minute period are increased, and this increase although definite is merely transient. The rate of glomerular filtration in the prodromal and B-avitaminotic animals has a pulsating character. The great initial filtration rate is replaced temporarily during the second 10 minutes by a slow filtration rate, this being followed by an increased filtration rate during the third 10 minutes.

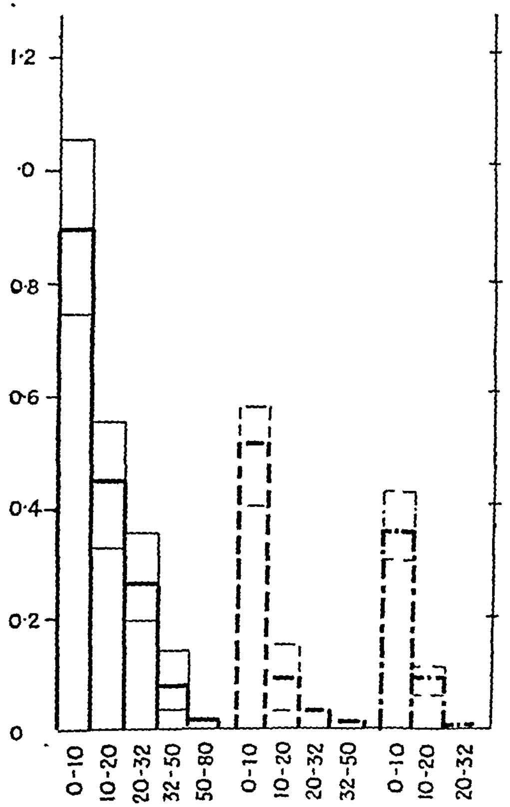

FIG. 5.—Curves for the sugar excreted in the urine during 10-minute periods by 5 normal dogs and by those in the prodromal and fully developed stages of avitaminosis. Abscissa: time in minutes after sugar-loading. Ordinate: amount of glucose in the urine, g./10 kg. body-weight. Solid lines: dogs in fully developed avitaminosis. Broken lines: dogs in the prodromal stage of avitaminosis. Dot-dash lines: dogs in the normal stage. Thin horizontal lines indicate the maximum and minimum values obtained; thick horizontal lines indicate the averages.

This conclusion regarding the pulsating character of the glomerular filtration in the prodromal stage and in the severely B-avitaminotic dogs is based on twelve observations. The pulsating nature was manifest in each and all of them. On the other hand, while these same dogs were normal, out of ten observations twice only was such a pulsating glomerular filtration found.

absorbing efficiency of the entire tubular surface decreases during B-avitaminosis, then the decrease of the absorbing power as a function of the increased sugar concentration in the ultrafiltrate must be greater than in the normal kidney. Contrasted with the normal, where the efficiency decreased to 88 per cent. from 94 per cent. when the sugar concentration of the ultrafiltrate decreased from 0·025 *M.* to 0·017 *M.*, in the B-avitaminotic kidney under similar conditions a decrease

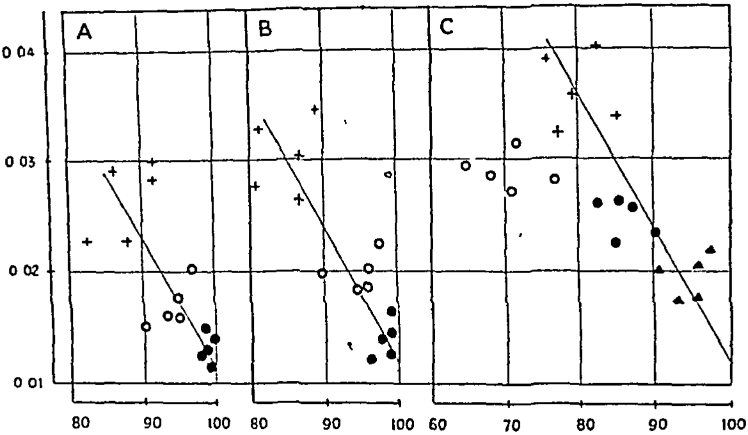

Fig. 6.—The relationship between the sugar-absorbing efficiency of tubular epithelium and molar concentration of sugar in the glomerular ultrafiltrate. Abscissa: percentages of sugar absorbed from glomerular ultrafiltrate. Ordinate: molar concentration of sugar in the glomerular ultrafiltrate. Crosses: results obtained during the first 10 minutes. Circles: during the second 10 minutes. Dots: during the 20–32-minute period. Triangles: during the 32–50-minute period of the experiment. A: normal. B: prodromal. C: fully developed avitaminosis.

greater than to 88 per cent. should take place, if there were a fall in the efficiency of the tubular epithelium. Consulting part C of fig. 6 (avitaminotic animals) the following may be seen. When the concentration in the ultrafiltrate was 0·035 *M.*, the efficiency was 80 per cent.; when the sugar concentration dropped to 0·030 *M.*, the efficiency was 70 per cent. Here, therefore, a very important contrast with the normal kidney is seen. In the normal kidney when the concentration in the ultrafiltrate decreases the absorbing efficiency increases; contrary to this, in the B-avitaminotic kidney with the decrease in the concentration, the efficiency also decreases. Our presumption regarding the decreased absorbing efficiency of the whole B-avitaminotic kidney is therefore justified. The situation, however, seems to be more complicated, because in the lower concentration range this decrease in the efficiency does not hold good: when the concentration decreases to 0·025 *M.*—as may be seen in the figure—there is no further decrease in the efficiency; on the contrary, the efficiency is almost as good as in the normal at the same molar concentration, viz. 88 per cent. This

We observed that the B-avitaminotic kidney excretes more sugar, and does so for a longer time than the normal kidney. It has also been observed that the concentration of sugar offered to the tubule surface for absorption from the glomerular ultrafiltrate is higher in the B-avitaminotic kidney than in the normal one.

This being so, a greater sugar excretion by the kidney will be the consequence even if the tubular reabsorption has not decreased. Indeed if we compare the absolute amounts of sugar reabsorbed in the B-avitaminotic and the normal kidney respectively, during identical periods, we find that the B-avitaminotic kidney takes up more sugar than the normal one, *e.g.* during the 20–32-minute period after loading the normal kidney absorbed 0·8 g., the B-avitaminotic one 2·38 g. of sugar. At this juncture it must, however, be recalled that during this same period different sugar concentrations were offered to the tubules. Thus in the normal ultrafiltrate the sugar concentration was 216 mg. per cent., whereas in the B-avitaminotic one it was 432 mg. per cent. in the 32nd minute of the experiment.

The circumstance that the absolute amount of sugar absorbed by the avitaminotic tubular epithelium is greater than that absorbed by the normal appears at first sight to contradict the supposition that the glucose-absorbing power of the tubules is decreased in B-avitaminosis. This supposition was based on the generalisation that other B-avitaminotic body-cells take up sugar at a slower rate. If the efficiency of the tubular epithelium in reabsorbing sugar is not decreased, then the increase in the sugar excretion by the whole kidney is entirely due to the greater sugar concentration in the glomerular ultrafiltrate, *i.e.* to extrarenal causes.

To decide this question we must obtain some information as regards the sugar-absorbing efficiency of the tubules, and we have first to discuss the relationship between the concentration of sugar in the ultrafiltrate and the absorbing efficiency of the tubules.

This may be done by plotting the absorbing power of the tubules against different sugar concentrations in the ultrafiltrate. As a measure of the absorbing power of the tubules the relationship—sugar in the ultrafiltrate : absorbed sugar—may be taken. And this for the sake of comparison we shall express as a percentage. The effect of sugar concentration on the absorbing efficiency of the tubular epithelium may be seen in fig. 6. It is evident from this figure that, in the normal kidney, as the sugar concentration in the ultrafiltrate decreases the absorbing efficiency of the tubular epithelium increases. The relationship appears to be inverse and linear. For instance, in fig. 6, A (normal animals) while the sugar concentration in the ultrafiltrate was as high as 0·025 *M.*, only about 88 per cent. of the sugar present in the ultrafiltrate was absorbed; when, however, the concentration decreased to 0·017 *M.*, 94 per cent. of the sugar was absorbed. If, therefore, the

Summing up this discussion it follows that the decrease in the absorbing power of the B-avitaminotic tubules is a consequence not of the alteration in filtration rate but of some intrinsic change in cellular activity. This conclusion is supported by the fact that the decrease in the efficiency of the tubules does not take place in the prodromal stage of avitaminosis, although the decrease in ultrafiltration rate in the whole kidney is well marked at this stage too.

SUMMARY.

1. In dogs in normal, in the prodromal, as well as in fully developed B-avitaminotic stages, the blood and urine sugar concentrations were determined after intravenous glucose injection. At the same time the rate of glomerular ultrafiltration was determined by the use of the creatinine clearance test.

2. It was found that the sugar content of the blood decreases more slowly in the prodromal and fully developed stages of B-avitaminosis.

3. The rate of ultrafiltration in the whole kidney of the normal animal shows a gradual decrease tending to reach an asymptotic minimum at the 50th minute. In both the prodromal and the fully developed stages of B-avitaminosis, the rate of glomerular ultrafiltration is not gradually decreasing towards an asymptotic minimum, but shows great variations. There is a big fall in the rate of ultrafiltration during the 10–20-minute period, and this is followed by an increase. During the experimental period there is no asymptotic minimum.

4. The sugar excretion in the whole kidney in prodromal and in fully developed B-avitaminosis is increased as compared with the normal.

5. The increased sugar excretion is due to two causes: an extrarenal and an intrarenal cause. The extrarenal cause is the greater sugar concentration in the glomerular ultrafiltrate—due to the slower sugar uptake of the body-cells; the intrarenal is a decrease in the efficiency of the tubular epithelium to absorb sugar from the ultrafiltrate.

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is also true in a third concentration range, viz. at a molarity of 0·020. The B-avitaminotic tubules, then, exhibit the remarkable property that, at a certain sugar concentration, their reabsorbing efficiency is much less than that of the normal one at about the same concentration. This is even more interesting because the efficiency of the B-avitaminotic tubules, both at higher and at lower concentrations, approaches the normal kidney rather closely. Therefore, we can safely conclude that the increased sugar excretion of the total B-avitaminotic kidney is due to a complex cause, composed of an extra- and an intrarenal component. The extrarenal component is the higher sugar concentration in the glomerular ultrafiltrate, the intrarenal is the decreased efficiency of sugar absorption by the tubular epithelium at certain concentrations of sugar in the glomerular filtrate.

The comparison of the rate of glomerular ultrafiltration in the kidney with the sugar-absorbing efficiency of the tubules produces an interesting question.

As is seen in fig. 4, the rate of glomerular ultrafiltration in both the prodromal and severe stages of avitaminosis shows variations. During the 10–20-minute period there is a fall which later is replaced by an increase. The decreased efficiency period of the tubules in B-avitaminotic dogs corresponds with that of decreased rate of glomerular ultrafiltration, and the possibility must be discussed whether the decreased absorbing capacity of the tubules is not due to the decreased rate of ultrafiltration. The rate of ultrafiltration was determined in the whole kidney. A fall in this rate may be the consequence either of a decrease in the number of active units or of a decrease in the rate of filtration in each of the working units. If the number of the working units is decreased, the relationship between the tubular cells and the ultrafiltrate in their lumina, in the remaining active ones, is not changed, and the decreased efficiency of the tubular epithelium must be due to an intrinsic change in cellular activity. If the decrease in the rate of ultrafiltration is the consequence of the decreased rate of filtration in each of the working units, then the question arises as to the influence of the velocity of flow of the filtrate in the tubular lumen on the rate of tubular absorption. Walker and Hudson [1937] presented evidence that in the frog the rate of glucose absorption by the tubular epithelium was inversely proportional to the velocity of flow of the ultrafiltrate in the tubular lumen. If it is permissible to apply their conclusion to our experiments, we should find an increase in the absorbing efficiency of the tubular epithelium, since during the 10–20-minute period the rate of ultrafiltration in the whole kidney was decreased; contrary to this, however, a decreased absorbing efficiency takes place during this period. The observed decrease in efficiency of the tubular epithelium must, therefore, be due to some intrinsic change in cellular activity.

CHOLECYSTOSTOMY FISTULÆ SUITABLE FOR SMALL LABORATORY ANIMALS.

By P. R. Peacock. From the Research Department, the Glasgow Royal Cancer Hospital.

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In the course of experiments on the metabolism of carcinogenic hydrocarbons [Peacock, 1936, and Peacock and Chalmers, 1936], samples of bile were collected from fowls, through simple fistulæ made by inserting a glass canula into the gall-bladder and connecting this with the extérior by means of rubber tubing. Such fistulæ were unsatisfactory for protracted experiments, owing to the small yield of bile obtained, and to the frequent supervention of cholecystitis. Operations involving drainage of bile into bottles or balloons outside the body were not attempted, as such contrivances interfere seriously with the freedom of the animals, and may introduce psychogenic disturbances of metabolism.

The following aims were kept in mind in evolving the technique to be described:—

(1) External appliances are undesirable, as they annoy the animal and are difficult to keep clean.
(2) The operation must not interfere seriously with the animal's metabolism.
(3) The fistula must be sterile.

Rabbits and fowls have been used in most of our experiments, and, as they differ greatly in their habits, the technique had to be modified for the two species, though the essential principle remained the same.

Methods.

A glass canula is inserted into the gall-bladder, and the bile is allowed to drain off, through rubber tubing, into a small rubber balloon (25–50 ml. capacity), which is placed either in the peritoneal cavity or in a pouch made in the subcutaneous tissues. The operation wound is completely closed, and no external dressings are applied. Based on this principle, operations have been evolved in this laboratory during the past four years, and a description of the technique now

silk or cotton. A second stab wound is made through the skin, at least 3 cm. above that through the abdominal wall, and the collecting tube is drawn through this. The skin is sutured in the same manner as the abdominal wall, and the end of the tube is marsupialised for about 2 cm. with two or three interrupted sutures. The arrangement of the balloon and collecting tubes is shown diagrammatically in fig. 1. At the end of the operation, bile-stained fluid can generally

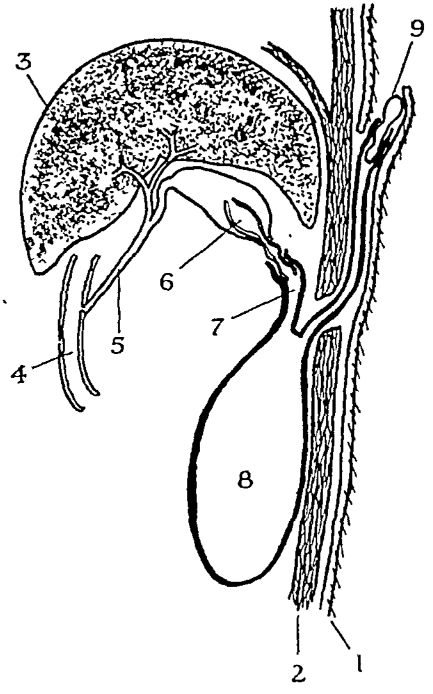

FIG. 1.—1. Skin. 2. Abdominal muscles and fascia. 3. Liver. 4. Duodenum. 5. Common bile duct. 6. Canula tied into gall-bladder. 7. 1st collecting tube. 8. Rubber balloon. 9. Glass plug closing 2nd collecting tube.

be expressed from the balloon by gentle pressure on the abdomen; this procedure also expels any air contained in the balloon. The collecting tube is then closed with a glass plug, and the skin is again painted with "spirit flavine." No further dressing is required, and the rabbits usually keep the wound and the end of the tube clean by frequent licking. Animals so treated make a rapid recovery and suffer no loss of appetite. Bile can be collected within a few hours of operation, but it is better to leave the animals undisturbed for twenty-four hours, as, after this time, they show no signs of discomfort. Thereafter, bile samples varying from 5 c.c. to 20 c.c. can be obtained daily, for about three to four weeks, after which time the yield generally diminishes. Samples are collected by removing the glass plug and

employed may therefore prove useful to other workers who require to collect frequent samples of bile from small laboratory animals.

The balloons are prepared by cutting off the rolled margin of the neck, and fixing into the balloon with rubber solution one or two collecting tubes made from bicycle valve tubing. When the solution has set firmly, the balloons are gently inflated through the collecting tubes, and tested under water for possible leaks. Leaking balloons are discarded.

Operative Technique.

Rabbits.—The hair over the abdomen is clipped with scissors and epilated with barium sulphide paste, about one hour before operation. As soon as the hair is loose, the skin is washed with soap and water, thoroughly rinsed and dried, and painted with a solution of acriflavine in equal parts of methylated spirit and water, to give a 1/1000 solution. Nembutal (75 mg. per kg. body-weight) is injected intramuscularly, followed by ether or chloroform, if necessary, to obtain full relaxation for manipulations of the gall-bladder. The skin is incised in the mid-line, from a point just above the xiphisternum, along the line of the linea alba for about 6 cm. The skin is rapidly separated from the muscles by blunt dissection, and sterile towels are clipped to the edge of the wound. With fresh scissors, the peritoneum is opened along the linea alba and the abdominal wall is retracted by two clips applied to the cut edges; traction may conveniently be made on these clips by small lead weights attached to them with light picture chain and allowed to hang over the sides of the operating table. The xiphisternum is raised by tissue forceps held by an assistant. This gives an excellent exposure of the liver, the superficial lobes of which can now be raised gently with a smooth retractor, to expose the gall-bladder, which is often deeply imbedded in the liver substance. The tip of the gall-bladder is seized by two pairs of small artery forceps. A suture is now passed round the gall-bladder by means of a cleft palate needle and a knot is loosely tied just below the tips of the forceps, without constricting the gall-bladder. The balloon is introduced deeply into the abdominal cavity, between the gloved fingers of the left hand, care being taken to avoid folding. The canula should now be in position, close to the gall-bladder, the tip of which is incised with fine-pointed scissors; the canula is slipped into the gall-bladder and immediately gripped by tightening the suture previously placed in position. This suture also closes the distal branches of the cystic artery and prevents hæmorrhage. A double knot secures the canula, which should now contain bile. Any free bile is gently absorbed with cotton-wool swabs. A stab wound is made through the left-flank muscles, and the collecting tube from the balloon is drawn through it. The abdominal wall is closed with continuous blanket sutures of fine

silk or cotton. A second stab wound is made through the skin, at least 3 cm. above that through the abdominal wall, and the collecting tube is drawn through this. The skin is sutured in the same manner as the abdominal wall, and the end of the tube is marsupialised for about 2 cm. with two or three interrupted sutures. The arrangement of the balloon and collecting tubes is shown diagrammatically in fig. 1. At the end of the operation, bile-stained fluid can generally

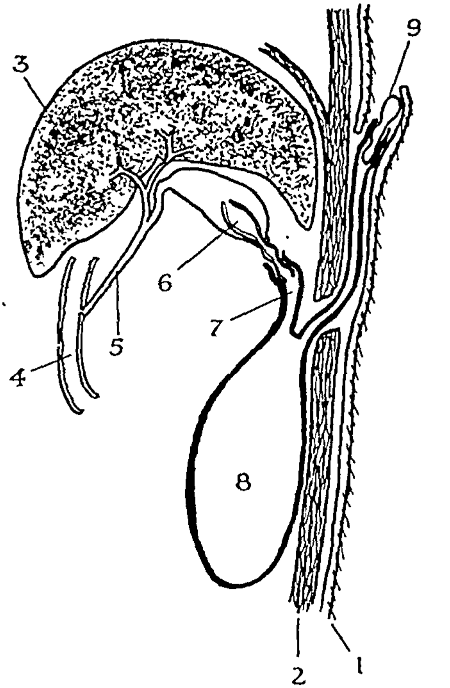

FIG. 1.—1. Skin. 2. Abdominal muscles and fascia. 3. Liver. 4. Duodenum. 5. Common bile duct. 6. Canula tied into gall-bladder. 7. 1st collecting tube. 8. Rubber balloon. 9. Glass plug closing 2nd collecting tube.

be expressed from the balloon by gentle pressure on the abdomen; this procedure also expels any air contained in the balloon. The collecting tube is then closed with a glass plug, and the skin is again painted with "spirit flavine." No further dressing is required, and the rabbits usually keep the wound and the end of the tube clean by frequent licking. Animals so treated make a rapid recovery and suffer no loss of appetite. Bile can be collected within a few hours of operation, but it is better to leave the animals undisturbed for twenty-four hours, as, after this time, they show no signs of discomfort. Thereafter, bile samples varying from 5 c.c. to 20 c.c. can be obtained daily, for about three to four weeks, after which time the yield generally diminishes. Samples are collected by removing the glass plug and

allowing the bile to run directly into a test tube; the process is facilitated by pressing gently on the abdomen. This method of collecting is preferable to aspiration with a syringe, which may cause sudden collapse of the gall-bladder on to the canula, followed by traumatic cholecystitis.

As this operation does not interfere with the common bile duct, the animal obtains sufficient bile to maintain it in good health indefinitely. The intraperitoneal balloons are tolerated remarkably

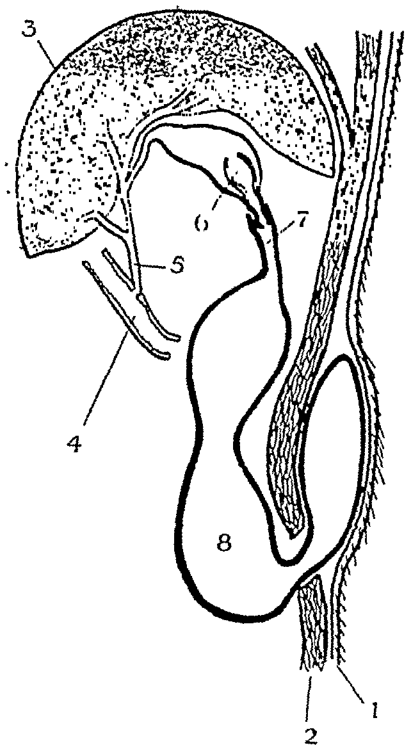

FIG. 2.—Nos. 1 to 8, as for fig. 1.

well, and we have rabbits in excellent health more than a year after operation, though the fistulæ have been allowed to close. Occasionally fistulæ remain open for several months, but, in most of our experiments, bile has been collected daily for about one month, and thereafter at less frequent intervals.

Recently, a completely closed operation has been used, with success (fig. 2). In this operation a toy balloon with numerous constrictions has been inserted into the peritoneal cavity, as described above, but, in place of a collecting tube, the terminal part of the balloon has been drawn through a stab wound in the rectus abdominis into the subcutaneous tissues and the skin completely closed. Bile is collected by

aspirating the contents of the terminal part of the balloon through the skin. This can be performed daily for many weeks without leakage of bile into the subcutaneous tissues, provided that small sharp needles (Nos. 14–18 hypodermic) are used.

Both these operations are satisfactory for rabbits, but the totally enclosed operation can more easily be kept sterile and is now used for preference in this laboratory.

Fowls.—On anatomical grounds, a somewhat different approach is necessary in the case of fowls. When the first operation is used, fowls peck at the collecting tube and tend to remove the glass plug. For this reason, a completely enclosed operation has been found more satisfactory for these birds.

The feathers are plucked over the right side of the sternum and abdomen, and the skin is swabbed freely with "spirit flavine." The best anæsthetic for fowls was found to be chloroform, given by blowing air through a modified Junker's apparatus. The skin incision is made along the lateral margin of the internal xiphisternal process, and is prolonged slightly towards the tip of the sternum. Blunt-pointed scissors are passed upwards through this incision in the subcutaneous tissues over the surface of the pectoral muscle to the level of the sternal notch, where a second small incision is made through the skin. The scissors are now opened widely and withdrawn in this position, leaving a space in the subcutaneous tissues for the reception of the collecting balloon. A small pair of pressure forceps is passed from the upper to the lower skin wound, through this space, and the balloon is grasped, drawn up into position, and secured by a suture passed through a small rubber tag fixed to its tip, and through the superficial pectoral muscle, as shown in fig. 3. The upper wound is closed, and sterile towels are clipped to the edges of the lower wound. The abdominal muscles are separated by blunt dissection and retracted to expose the peritoneum. The internal xiphisternal process and the attached muscles are grasped with tissue forceps, which are held vertically by an assistant. The peritoneum is incised, and the gall-bladder can usually be seen, though it is sometimes overlapped by the liver. A stab wound is now made through the abdominal muscles, a little below the centre of the wound, and the canula and collecting tube are passed through this into the peritoneal cavity. The canula is inserted into the gall-bladder, in the same manner described for the operation on rabbits. The abdominal wall and the skin are then closed with continuous blanket sutures, and the skin is again swabbed with "spirit flavine." This operation should be practically bloodless, as the subcutaneous vessels in the fowl can be seen through the skin, and avoided. The subcutaneous balloons are well tolerated, and may remain in position for a year or more, though, occasionally, pressure atrophy of the skin occurs, and the whole balloon may be pulled out by the bird. Bile is collected by

aspirating the contents of the balloon through the skin, using a hypodermic syringe and a No. 14 needle. Daily puncture of the balloon

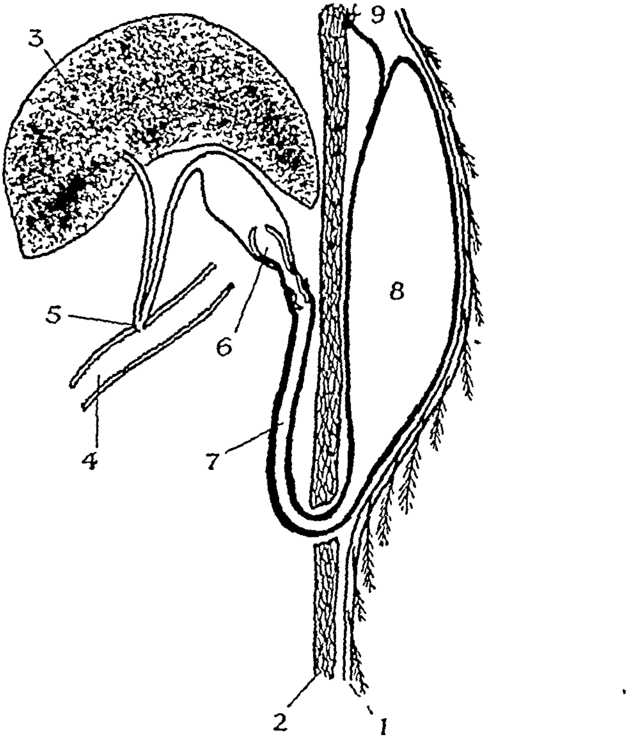

Fig. 3.—Nos. 1 to 8, as for fig. 1. No. 9. Tag attached to balloon and sutured to muscle.

may be made in this way for several months without leakage of bile occurring into the subcutaneous tissues.

Results.

Most of our animals received intravenous injections of benzpyrene colloid at frequent intervals, and cannot therefore be considered as normal. Nevertheless, the bile yields were comparable in volume and specific gravity with those from untreated control animals. Tables I. and II. give the relevant data for a typical normal fowl and rabbit.

Variability in yield is probably due to the fact that the common bile duct was not tied, and the volume of the bile collected does not therefore give a measure of the total bile excreted. Body-weight is not recorded in every case, but the health of the animals was excellent, and where weights were recorded they remained steady, or increased, during the period of observation.

Table I.

Animal.	Date 1940.	Body-weight, g.	Food consumed, g.	H_2O, ml.	Excreta, g.	Bile. Vol., ml.	Specific gravity (H_2O/1000).	Total solid, g./100 ml.	Ash, g./100 ml.	Remarks.
Fowl No. L.3319. Operation—8th April 1940.	Apr. 9					7·5	1007	4·3	0·8	Dark green.
	10					5·0				Culture sterile.
	13					5·0	1004			Dark green.
	15									
	16	1000	79	58	31	3·0	1009			,,
	17	1000	75	56	35	5·0				,,
	18	975	72	61	30					,,
	19	960	74	61	36	4·0	1004			,,
	20	960	66	60	27					,,
	21	975	68	84	21					,,
	22	1000	68	54	35	3·0	1007	2·3	0·53	,,
	23		72	59	28					,,
	24		70	58	35					,,
	25		40	75	17	5·0				,,
	26	990	50	60	27	2·0	1006			,,
	27	990	55	74	18	2·0				,,
	28	990	62	58	39					,,
	29	1000	62	66	45	2·0				,,
	30	1000	50	60		2·0				,,
	May 1	990	56	62	25	2·0				,,
	3					2·0				,,
	4		50	62	15	2·0				,,
	22					10·0				Culture sterile.
	May 22 to July 29	1125	40	75		4·5 *	1005			Left undisturbed for one month, 29th July to 28th August.
	Aug. 28	1430	98	180	98	10·0	1009			Dark green, sterile.

Food consumed = Mixed grain. * = Daily average.

The specific gravity and total solid, and ash estimations were performed by Mr. A. H. M. Kirby in this Department. The specific gravity of different bile samples has varied between 1003–1010 for rabbits, and between 1003–1009 for fowls.

Discussion.

In our experiments the type of enclosed collecting system has only been applied to rabbits and fowls, but there seems no reason why this type of operation should not be used in other laboratory animals, particularly where intubation of the bile ducts is not practicable owing to their small size.

The advantage of this type of operation, in our experience, has been

the total absence of dressings or external appliances, and the excellent health of the animals. All animals are very intolerant of dressings, and it may be questioned whether the secretions of animals confined in such a way that they cannot interfere with dressings can be regarded as normal.

TABLE II.

Animal.	Date 1940.	Body-weight, g.	Food consumed, g.	Fæces, g.	Urine, g.	Bile.				
						Vol., ml.	Specific gravity (H_2O/1000).	Total solid, g./100 ml.	Ash, g./100 ml.	Remarks.
Rabbit No. 1536. Operation—12th April 1940.	Apr. 14					5·0	1003	1·9	0·25	Brownish green.
	15					12·5	1007			,,
	16					12·0	1004			,,
	17					10·0	1004			,,
	18					5·0				Brown, with reddish tinge.
	19					9·0	1005			Clear brown.
	20					9·0	1005	5·3	1·4	,,
	22					5·0	1009			,,
	23					5·0	1009			,,
	24					5·0	1004			,,
	25					7·5	1010			Clear green.
	26	2000	450	32	116	5·0				Pale green.
	27	2000	450	19	156	5·0	1008			,,
	28	2000	450	21	144					,,
	29	2000	450	22	172	8·0				Pale green. Few fibrinous shreds.
	30	2010	450	31	260	7·5				Pale green. Trace of blood.
	May 1	2000	450	24	309	4·0				Pale green. Skin over balloon ulcerating.
	2									Killed.

Food consumed = Greens.

SUMMARY.

Operations are described for the collection of daily samples of bile from rabbits and fowls:

(1) In the operation for rabbits, a collecting balloon is connected with the gall-bladder by means of a canula and rubber tubing, all of which are placed in the peritoneal cavity. A second collecting tube leads from the balloon to the surface, through a stab wound (fig. 1).

Alternatively, a collecting balloon of special shape is placed partly in the peritoneal cavity and partly in the subcutaneous

tissues; bile is collected by aspirating the contents of the subcutaneous part of the balloon through the skin (fig. 2).

(2) In the case of fowls, the collecting balloon is placed in the subcutaneous tissues, and is connected with the gall-bladder by means of a single rubber tube and canula. Bile is collected by aspirating the contents of the balloon through the skin (fig. 3).

Rabbits and fowls both yield bile samples of about 5 ml. to 15 ml. daily, for about four weeks after this operation, and remain in good health for periods of a year and more.

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ON THE SURVIVAL OF THE TRANSFUSED ERYTHROCYTES OF STORED BLOOD. By Patrick L. Mollison and I. Maureen Young. From the S.W. London Blood Supply Depot.

(*Received for publication 30th September* 1940.)

The opinion that the erythrocytes of stored blood do not survive long after transfusion has been expressed by many authors; thus Riddell [1939] considers that because of the increased fragility of the cells of blood that has been stored for more than four days, "it is very doubtful whether it would exert any prolonged therapeutic effect." Marriott and Kekwick [1940] sum up available evidence as follows: "Red cells of blood conserved for more than a week by present methods do not last long in the recipient." Again, Edwards and Davie [1940] state: "Aged erythrocytes and those in which the fragility has been increased have only a short effective span of life and can therefore have little lasting value."

These statements are, perhaps, rather surprising, since in 1916 Peyton, Rous, and Turner [1916], from their experiments on rabbits, concluded that cells stored up to two weeks function normally after transfusion. This conclusion was reached after studying blood counts and estimating the excretion of hæmoglobin and bile pigments, etc.

In March of this year we had the opportunity of examining some results obtained by Dr. J. O. Oliver, then Director of this Depot, and Dr. G. L. Taylor of the Galton Laboratory Serum Unit. They followed seven cases by the direct differential method (referred to below), using M and N agglutinating sera. They were able to show that transfused erythrocytes of stored blood survived for some weeks after transfusion. In one patient, for instance, who received 11-day-old blood the donor cells could be identified by direct agglutination after 51 days.

The object of this investigation has been to extend these observations further and in greater detail and to apply to the problem a method capable of giving quantitative results. Methods of following quantitatively the fate of transfused erythrocytes are all based upon the method described by Ashby [1919 *a*]. This, in brief, consists of the transfusion of cells of Group O to a recipient of Group A. After transfusion the recipient's blood contains two types of cells, O and A. The A cells can be "removed" with alpha serum and the free (O) cells

counted. Actually a small fraction of the A cells are not agglutinated, and constitute a blank value to be deducted from the total of free cells.

Wiener [1934] has adopted the method using the sub-groups M and N. For example, if cells of Group M are transfused to a recipient of Group N, the N cells can be agglutinated and the free (M) cells of the donor counted. Considerable criticism has been levelled both at the general validity of these methods and also at the reliability of the technique. Isaacs [1924] suggested that following transfusion a number of immature cells would be thrown into the circulation, and being, as he thought, inagglutinable, would simulate donor cells. Ashby [1924], however, showed that the extent of the rise in the inagglutinable count depended upon the quantity of blood transfused and upon the patient's body-weight. Furthermore, this rise did not occur when a patient of Group A received a transfusion of Group A blood but only when Group O blood was transfused.

In addition, if blood of Group M is transfused to a patient of Group N a further refutation of Isaacs' theory may be made. Before the transfusion, agglutination with anti-N serum will leave free only a small fraction of the patient's cells—the blank "inagglutinable count." Following the transfusion a greatly increased number of free cells will be found. If now a sample of the recipient's blood is agglutinated with anti-M serum in addition, it will be found that this excess is "removed" and only a small fraction of free cells, equivalent to the initial blank figure, is left. We have performed this experiment several times and always with the same results.

The reliability of the technique has been carefully considered by Jervell [1924], who reached the conclusion that the method of Ashby could yield important quantitative results. He considered that the error of the method was plus or minus 10 per cent., but was careful to point out that the potency of the sera used and the effects of temperature, concentration, etc., are of great importance. A convincing demonstration of the quantitative nature of the results may be made by performing agglutination of the same cells with two different sera (viz. of the ABO and MN systems). A description of such an experiment is given in the Appendix.

The object of the work here described has been primarily to perform quantitative estimations of the numbers of donor erythrocytes surviving at various periods after the transfusion of stored blood. In addition an attempt has been made to support the contention that the figures have a quantitative significance.

The Blood used for Transfusion.

In every case blood was taken from supposedly healthy donors into a diluent consisting of 1·05 per cent. sodium citrate in 0·85 per cent.

saline. 360 c.c. of blood were taken into 180 c.c. of this diluent. Sufficient glucose was added to the citrate saline mixture after autoclaving to provide a final concentration of 1 per cent. in the blood mixture.

Since many transfusions were of the O to A type, a suspension of cells only was used to avoid the possible transfusion of a large bulk of high titre agglutinins. This can be very conveniently accomplished since stored blood sediments quite rapidly and the supernatant plasma may be removed. In most cases the cells were resuspended in 1·1 per cent. saline with 1 per cent. glucose as this solution produces minimal hæmolysis [MacQuaide and Mollison, 1940]. In a few cases the cells were given in a minimal quantity of plasma. Different quantities of this cell suspension were given to different patients, but usually the equivalent of two bottles of stored blood (*i.e.* 720 c.c. of whole blood) was given. The blood was stored at 4° C. for various periods (1–29 days) before transfusion. The blood was usually given at approximately 3–10 c.c. per minute.

Clinical Material.

The majority of the patients transfused were suffering from anæmia connected with pregnancy or parturition. A certain number were delivered whilst they were still under observation, and an indication of this is given in the figures.

Methods.

The experiments can be divided into three groups:

1. Simple O to A or M to N transfusions.

The results of these experiments have been combined in figs. 1 and 2.

2. A combination of the above two methods in the same patient. For example, patient A. C. belonged to Group BN, and was transfused with blood of Group OM. The donor erythrocytes could be counted after agglutination of the recipient's cells either with beta serum or with anti-N serum, neither of these sera agglutinating the donor erythrocytes. The parallelism between the two sets of figures is striking. We consider that the three cases followed in this way afford strong evidence of the quantitative validity of our other results. Martinet [1938] used a similar method as a check upon his experiments, using the M and N sub-groups. However, his experiments were only qualitative.

As mentioned above, we also applied this method to our *in vitro* experiments, and an example is given in the Appendix.

3. The measurement of the survival of two different transfusions in the same patient:

(*a*) Transfusions of two different "ages" of blood.
(*b*) Transfusions of blood stored in two different ways.

These experiments are in the nature of controls.

The simplest method illustrated by the patient E. (see fig. 4) is to give a second transfusion at a sufficient interval after the first and when all the cells of the first transfusion have been destroyed.

This method involves observing a patient for many months, and the patient's ability to deal with the transfused cells may have altered in the meantime. To avoid these difficulties, if the recipient is of Group AB two transfusions may be given simultaneously and followed separately. This can be most simply done by giving one transfusion of Group A and one of Group B. It may also be done by giving some blood of Group O and some of Group A; thus, in the patient D. S., a transfusion of 4-day-old blood of Group O was followed by a transfusion of 21-day-old Group A. After these two transfusions agglutination with beta serum left free the cells (A plus O) of both transfusions. With alpha serum only the O cells of the first transfusion was left free. By simple arithmetic the number of A cells surviving could be counted.

Since persons of Group AB do not amount to more than 3·2 per cent. of the London population [Wiener, 1939], a more generally applicable method is the use of the M and N sub-groups in addition. Thus, if a patient of Group AM is transfused (i) with OM blood, and (ii) with AN blood, agglutination with alpha serum will leave free the OM cells of transfusion (1), and agglutination with anti-M sera will leave free the AN cells of transfusion (2).[1] The two transfusions can be given immediately one after the other (a little saline being washed through the apparatus in between).

Although we have performed all our experiments quantitatively, we have in some cases used the method of "Direct differentiation" [Dekkers, 1939] to determine the "end-point." This method consists in the identification of the donor cells by direct agglutination, using the M and N sub-groups. Thus, when the number of inagglutinable cells falls to a figure which is but little higher than the original blank value, a decision as to whether or no donor cells are still surviving can be made by testing for the presence of the foreign agglutinogen. Such a combination of methods was first suggested (but not applied) by Martinet [1938].

As an example, an asterisk has been inserted in fig. 6. At this point, 84 days after the transfusion only 45,000 cells (per bottle) could be attributed to the donor by the quantitative method, and one cannot be certain that this number exceeds the possible variations in inagglutinable count. A test for the agglutinogen M was therefore made, the recipient and the donor of the second transfusion both belonging to Group N. The finding of small agglutinates showed that the cells of the first transfusion were still surviving.

Technique.

Since we did not adhere to the details of the technique originally described by Ashby, we feel that it is necessary to give our reasons for the changes which we adopted.

Briefly, Ashby's technique consists in taking blood into a leucocyte

[1] Since the completion of this work, we have learned that Wiener and Peters [1940] used this identical method to compare the survival of two transfusions in the same patient, though for a different purpose.

pipette and then drawing up agglutinating serum so that a final concentration of blood/serum of 1/22 is obtained. The mixture is expelled into a tube and incubated (with shaking at intervals) for 40 minutes. The tube is placed in an ice-box overnight and then counts of the free cells in the fluid are performed.

Our criticisms of this technique fall under four headings:

(1) The concentration used. A concentration of 1/22 makes blood-counting very difficult when more than 600,000 cells per cu. mm. are present. Furthermore, Rouleaux formation is definitely commoner at this concentration and further interferes with counting.

(2) The temperature suggested for incubation is 37° C. This is difficult to understand, since it is well known that warming hinders agglutination. Jervell, too, was puzzled by Ashby's choice of temperature, and actually performed his agglutinations in a refrigerator. This, however, is open to the disadvantage that it provides an opportunity for the action of cold agglutinins (as noted later by Ashby, 1919 *b*). We therefore performed our experiments at room temperature.

(3) Forty minutes does not seem to be an adequate time for agglutination. Taylor [1938] suggests that two hours is the optimum.

(4) It is well known that flat-bottomed vessels favour agglutination, and therefore the tubes recommended by Ashby were replaced by small bottles of such a size that the amount of fluid (0·2 c.c.) formed a thin film upon their floor.

With these considerations in view we adopted the following technique: 20 cu. mm. of the blood to be tested was taken from the patient in a hæmoglobin pipette and blown into 1 c.c. of citrate saline. From this suspension (1/50) 0·1 c.c. was taken and mixed with 0·1 c.c. of the appropriate serum in a small flat-bottomed bottle (as used for performing a red cell count). (0·1 c.c. only was used simply because large quantities of reliable anti-M and N sera are not available.)

The mixture was left for 2 hours at room temperature with brief shaking at half-time. At the end of 2 hours the bottle was shaken vigorously and a drop of the fluid withdrawn and placed in a Burker-counting chamber. This chamber was chosen because of the large area of the ruling. This enabled a selection of columns to be made, when large agglutinates obscured part of the field.

All the experiments were performed in duplicate, and counts in every case were made by both of us simultaneously.

To determine what constitutes an "agglutinate" is not easy, particularly when pairs are seen. We found it helpful to examine control experiments in which no agglutinating serum was present. This enabled the pairs which were stuck together to be distinguished from those that

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follow, this net total was then divided by the number of bottles of stored blood given to the patient.

The initial figure after transfusion depends upon many factors:

(1) The donor's red cell count and the volume of blood transfused.
(2) The recipient's blood-volume.
(3) The amount of destruction of donor cells that has already occurred before the sample is taken.

The first factor was partially standardised, as mentioned above, by dividing the number of surviving cells by the number of bottles of blood given.

We hoped to minimise blood-volume changes by using concentrated suspensions of red cells. Although the commencing destruction of

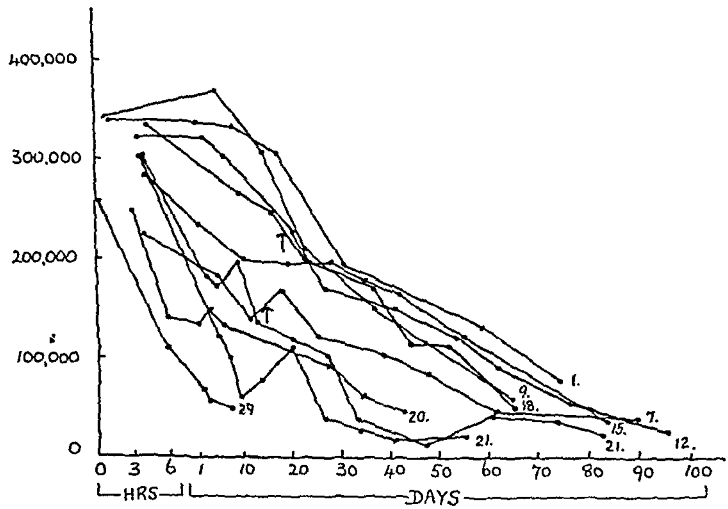

FIG. 1.—Survival of single transfusions of stored blood. The figures at the end of the curves indicate the length of storage in days. The initial figure depends upon many factors (discussed in the text), but after taking these into account, the slope is the important index of survival.
An arrow indicates that at this point the patient was delivered of a child.

effete red cells introduces a certain error, yet if the first sample is obtained within three to four hours of the mid-point of the transfusion, the expected number (approximately) is found except with blood more than three weeks old. (Example below.)

From fig. 1 it will be seen that, on the whole, the figures fall upon an even slope, and further that this slope is much the same with all the blood stored for less than 18 days. However, with the older blood the destruction is more rapid.

In two cases in which blood of 20 and 29 days old respectively was given, different samples were taken within the 6 hours following transfusion, and it was found that that period is a time of rapid destruction.

are agglutinated. Discoloration of cells is always held to indicate agglutination. When the difference in parallel experiments was greater than expected the counts were repeated, and the pair showing the best agreement were selected. When any gross discrepancy was found agglutination was performed again.

Görl [1926] has objected that shaking may increase the number of free cells considerably. This is not supported by a theoretical consideration of the subject, for the attractive force between agglutinated cells is very great. Moreover, we have found that moderate shaking for fifteen seconds or less does not significantly raise the number of free cells. Furthermore, we have frequently found very few inagglutinable cells after vigorous shaking. Jervell advises shaking for one minute, and found no significant rise even after two minutes' shaking.

The titre of the serum was found to be of the greatest importance in securing consistent results. We were fortunate in obtaining sera of very great potency from Dr. G. L. Taylor of the Galton Laboratory Serum Unit. The alpha serum used throughout had, for example, a titre of 1/256, and the beta of 1/512.

We have found that cells of the Sub-group A_2 are difficult to agglutinate completely with ordinary grouping sera. Even with sera of high titre the "inagglutinable count" of A_2 cells is usually higher than that of Group A_1. Therefore A_2 cells provide a useful control in the selection of a suitable alpha serum. An alpha serum that will give an inagglutinable count of less than 60,000 cells per cu. mm. with Group A_2 blood may be considered satisfactory. Higher figures previously recorded for the blank "inagglutinable count" are mainly, we suggest, due to working with sera of insufficient potency.

The M and N agglutinating sera are not, on the whole, so reliable as those of the ABO system—presumably because they are "absorbed" sera. Thus some samples prove to be of insufficient potency to produce complete agglutination and others give non-specific reactions. The latter can usually be avoided by dilution with an equal volume of saline, and we adopted this precaution throughout and also worked with controls. Some patients were followed with three different sera, but eventually we worked only with one reliable anti-M and one anti-N, and the experiments in section (3) show the good quantitative agreement with the results obtained with alpha and beta sera.

Results.

We have expressed our results graphically, plotting the number of cells surviving against the time since the transfusion. In every case we performed agglutination on a sample of the patient's blood before transfusion, and subtracted this "blank" value obtained from the number of free cells found after transfusion. In the figures which

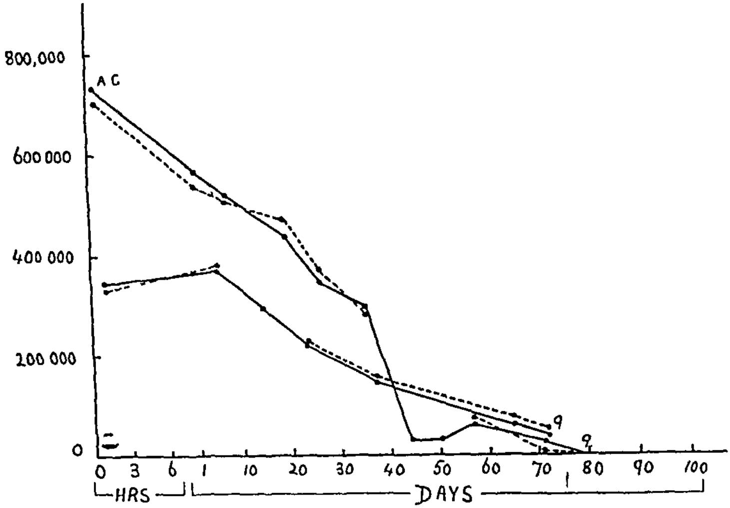

FIG. 3 —Survival of two single transfusions given to different patients. The continuous lines show the figures found when agglutination was performed with sera of the ABO system; dotted lines indicate those found with the MN sera. Note the good agreement. (Patient A. C. was a child.)

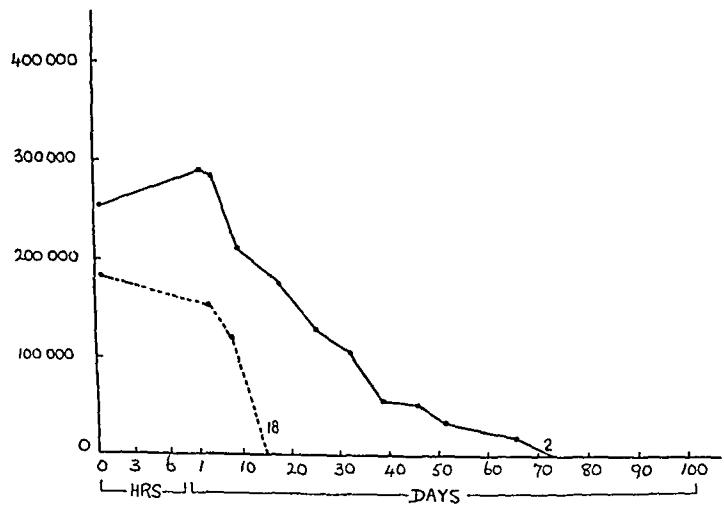

FIG. 4 —Survival of two transfusions given consecutively to the same patient. Note that the survival of the 18 day-old blood is very short, and the survival with 2 day-old blood is definitely less than expected.
The patient was suffering from an undiagnosed variety of reticulosis.

splenomegaly, and was jaundiced before the transfusion. Since in this case the destruction seems to have been more rapid than in the others, it may certainly be rash to draw conclusions about the life of transfused

The fact that the transfusion of stored blood is followed by an immediate rise in bilirubin agrees well with these observations. For instance, in the patient who received 20-day-old blood (see fig. 1), 3 hours after the transfusion 594,000 cells per cu. mm. attributable to the donor were found and the bilirubin had already risen from 0·8 to 3·0 mg. per cent. Six hours after the transfusion only 274,000 cells per cu. mm. remained, and the bilirubin had risen to 4·5 mg. per cent. Six days later, however, there were still 250,000 donor cells per cu. mm. It should be pointed out that this is a considerably greater initial destruction than occurred in the majority of the cases.

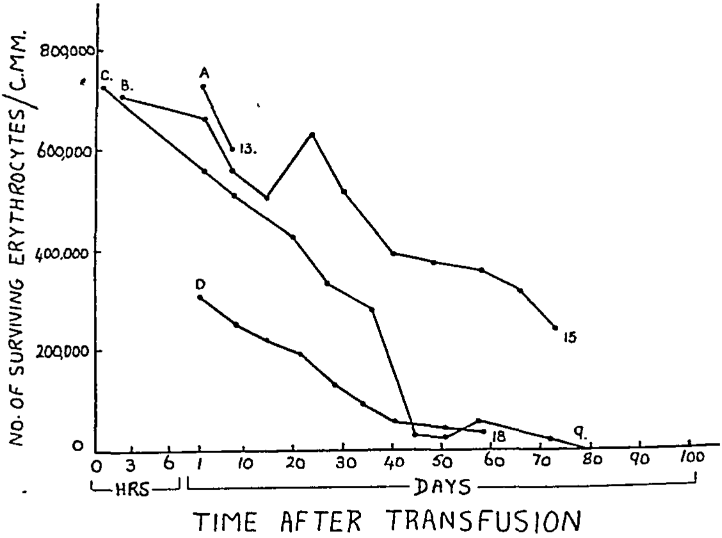

Fig. 2.—As fig. 1, but recipients all children. Note greater rise per bottle of stored blood.
Weight of children: A., 3 stone; B., 3·5 stone; C., 4·0 stone; D., 4·5 stone.

The total life of the transfused erythrocytes of blood stored for less than 18 days appears to be at least 70 to 90 days.

Fig. 2 shows that much greater rises in the number of donor cells per cu. mm. are produced by equivalent amounts of blood when given to children. Furthermore, the rise produced is approximately inversely proportional to the body-weight. The life in two of these four cases seems to be a little shorter, viz. practically all donor cells have disappeared at 60 days.

Fig. 3 shows the good agreement between the results of agglutination of recipient cells with two different sera. Fig. 4 shows the survival found after two transfusions of blood of different ages. Although the 18-day-old blood was all destroyed in 15 days, it will be seen that the life of the 2-day-old blood was only about 75 days, that is to say, definitely shorter than normal. This patient had in fact hepato-

each case. It will be seen that not only is there little difference between the survival of the different ages of blood, but that the curves suggest

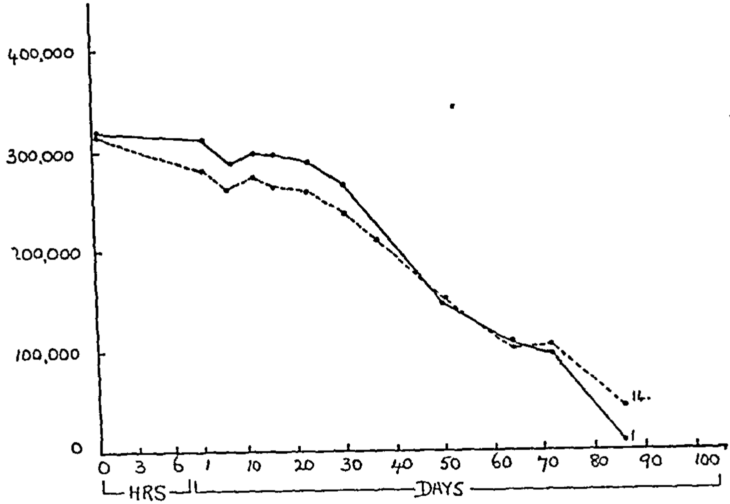

Fig. 7.

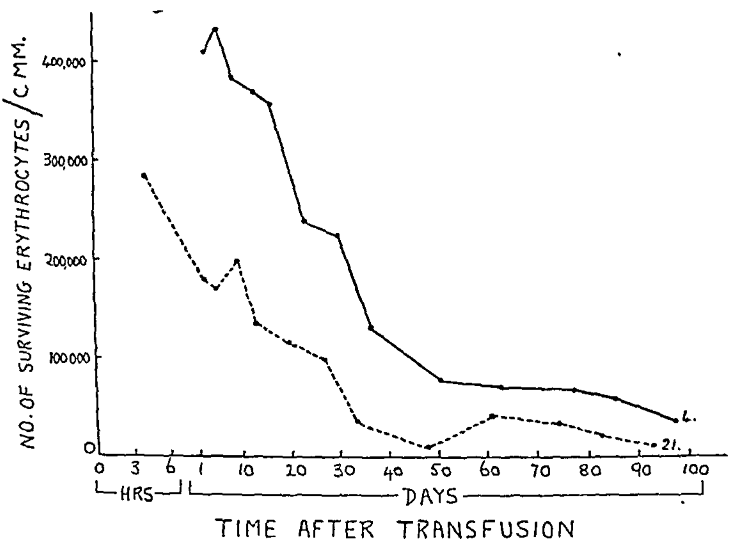

Fig. 8.

Figs. 5–8.—In each case the figure illustrates the survival of two different transfusions given simultaneously to the same patient. Storage for 16 days appears to have remarkably little effect upon subsequent survival.
In fig. 6 the asterisk indicates that a direct test for the donor agglutinogen was positive at this point.

that the life is practically as long as that of fresh blood until blood of over 16 days old is used (see fig. 8).

erythrocytes from a few cases. Very much greater certainty is giv
to the conclusions by the results in the following section of the worl

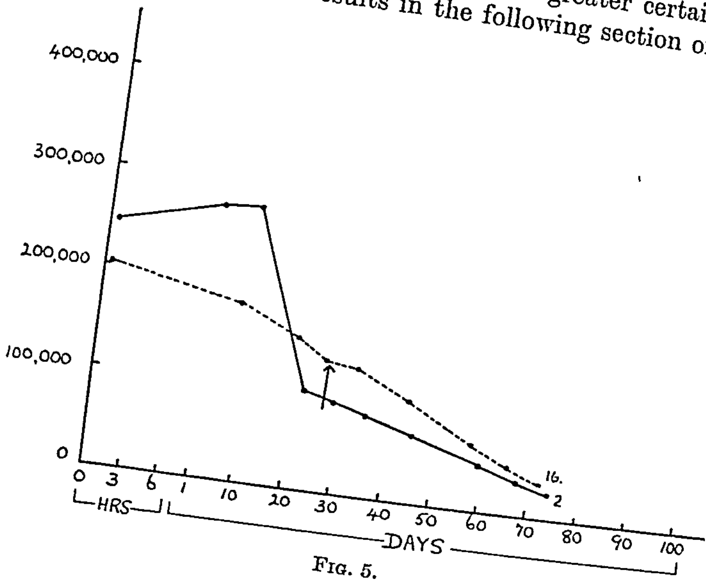

FIG. 5.

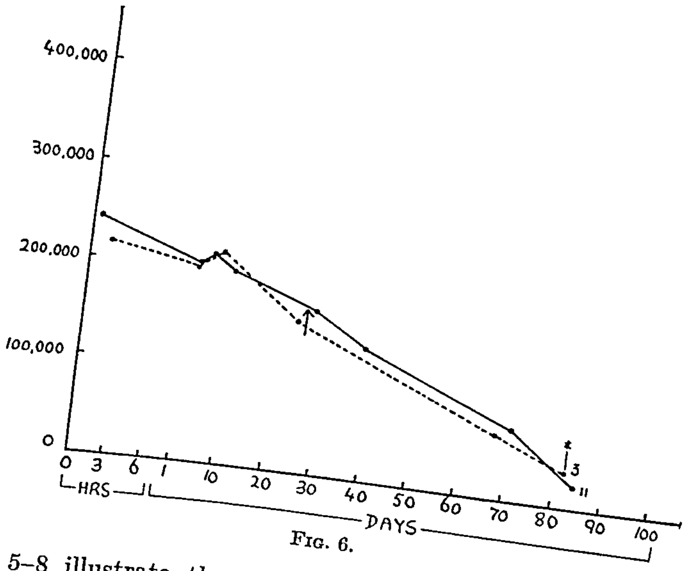

FIG. 6.

Figs. 5–8 illustrate the comparison of the survival of blood of
erent ages, two simultaneous transfusions having been given in

differences in the technique of taking blood and in the patients' treatment of the transfused cells. It seems more probable, however, that they are to be accounted for by the rather rough quantitative method employed.

The greatly inferior survival of the cells of blood stored with citrate compared with that of the cells of blood stored with glucose in addition might have been anticipated from a study of the results of *in vitro* experiments on stored blood. Rous and Turner [1916] first showed the value of glucose as a preservative for red cells, and numerous investigators have confirmed their results in recent years.

Discrepancies in the life of stored blood will probably be reported until the optimal preserving solution and the optimal dilution for the storage of blood are agreed upon.

Summary.

Twenty patients have received 26 transfusions of stored blood, and the fate of the erythrocytes of the donor followed quantitatively by differential agglutination.

A method of performing control agglutinations using the sera both of the ABO and MN systems is suggested.

A further application of differential agglutination is outlined. Its purpose is the following of the fate of two transfusions given simultaneously. This method has been used for (1) comparison of methods of storage; (2) to obtain a comparison of the survival of two different "ages" of blood in the same patient.

Conclusions.

1. A high proportion of the erythrocytes of blood stored in citrate-saline-glucose survive transfusion. Furthermore, the total time of survival is little inferior to that of fresh blood.

2. These observations suggest that the present method of storage is good and that little more than the normal ageing process occurs at least in blood stored for 18 days or less.

In connection with Ashby's work on the survival of the transfused erythrocytes of fresh blood, P. E. Weil [1940] has remarked: "Ce n'est donc pas aux depéns de l'hæmoglobine des globules détruits que l'organisme trouve le possibilité de former des nouveaux globules, mais ce sont bien les globules transfusés eux-même qui continuent leur vie et leurs fonctions physiologiques."

These words might be used as a refutation of the remarks quoted at the beginning of this paper.

The importance of the method of storage is emphasised in fig. 9, which shows a comparison between the survival of blood stored with dextrose and blood stored with dextrin. A transfusion of each variety of blood was given to the same patient and followed by the method we have outlined in section (3) (above). It will be seen that the survival of the blood stored with dextrin is definitely inferior to that of the blood stored with dextrose.

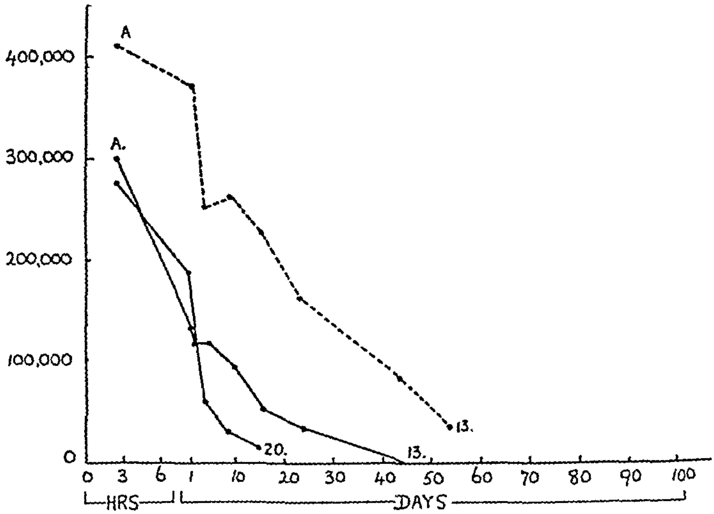

FIG. 9.—The continuous lines mark the survival of two transfusions of blood preserved with dextrin and given to different patients.
Patient A. also received simultaneously a transfusion of blood stored for the same length of time, but in dextrose. The survival of this transfusion is indicated by a dotted line. It is clear that, firstly, the survival of the cells preserved in dextrin is less than that of the cases of blood of equivalent age stored in dextrose, and secondly, that in the direct comparison blood stored with dextrose has an obviously greater survival.

It is interesting to compare these results with those obtained by Wiener and Schaefer [1939, 1940]. These workers gave transfusions of stored blood to 28 patients and followed the survival of the donor cells by a rough quantitative method. The blood was kept with sodium citrate only.

The survival was found throughout to be much shorter than in this work. For instance, two patients received 13-day-old blood. The time for total disappearance of donor cells was found to be 56 days in one and 42 days in the other recipient. The authors suggest that each day of storage reduces the survival by about 6 days.

The results, although showing a general inverse correlation between length of storage and subsequent length of survival, are very variable and give little information about the intermediate "curve of destruction." The authors suggest that the variations are accounted for by

This figure may be compared with one given by Ponder [1934] (for ordinary blood-counting). According to this author, when 500 cells only are counted, two counts must differ by more than 12 per cent. before the difference can be considered significant.

As only 500 cells were counted on each occasion in this experiment it will be seen that the error is of the same order as that of ordinary blood-counting.

We wish to thank Dr. J. O. Oliver, former Director of this Depot, and Dr. O. M. Solandt, his successor, for advice and criticism, and also Dr. M. Maizels, who provided the impetus for this work and who was generous enough to allow us access to his results at all stages.

We should also like to thank Dr. G. L. Taylor of the Galton Laboratory Serum Unit for providing M and N agglutinating sera, without which this section of the work could not have been done.

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APPENDIX.

In vitro Experiment to Determine Accuracy of Counting Cells of Group "ON" in the Presence of Cells of Group "BM" after Agglutination with (1) Beta Serum, (2) Anti-M Serum.

Description.—A suspension of "ON" cells was prepared and two dilutions of it were made. To each dilution was added an equal volume of a more concentrated suspension (1/50) of "BM" cells. 0·1 c.c. of this solution was mixed (1) with 0·1 c.c. of beta serum, and (2) with 0·1 c.c. of anti-M serum, in small bottles (see description of method in text). After two hours, counts of the free cells were made. In all, 31 counts were performed.

By counting the suspension before mixing, the expected number of free cells could be calculated.

Expected.	Found.	
	With beta serum.	With anti-M serum.
1,134,000	1,195,000	1,195,000
	1,200,000	1,227,000
	1,189,000	1,179,000
	1,218,000	1,138,000
	1,189,000	1,139,000
	1,188,000	1,198,000
	1,189,000	1,195,000
	1,248,000	
577,000	558,300	550,800
	565,800	554,100
	576,700	545,000
	547,400	573,300
	554,200	542,700
	585,000	556,000
	575,000	557,500
	570,000	551,700

By agglutinating with BM cells only, blank values were obtained, viz. (1) with beta serum 20,000 cells per cu. mm. (av.), (2) with anti-M serum 22,000 cells per cu. mm. (av.). These figures have been added to the number of free cells to be expected.

It will be noticed, firstly, that agglutination with beta and anti-M sera give closely similar results. Secondly, it will be seen that the "scatter" of the figures is small, but that in the first dilution all the figures are greater than expected, and in the second dilution they are, with one exception, lower. These errors presumably arise in performing the dilutions and, therefore, are liable to occur in any experiments made with this method.

The coefficient of variation in this experiment was found to be approximately 5 per cent., that is, two counts must differ by at least 10 per cent. to be significantly different.

THE INFLUENCE OF THE VAGUS ON THE SECRETION OF MUCUS BY THE STOMACH. By M. A. Jennings [1] and H. W. Florey. From the Sir William Dunn School of Pathology, Oxford.

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The secretion of mucus by the stomach occurs under both physiological and pathological conditions—especially the latter, in which some form of "irritation" is usually present. The influence of the nerves on this mucus secretion is not yet quite clear. For many years it has been recognised that the fundal secretion—*i.e.* HCl and pepsin—is partly controlled by the vagus. Recently Wright *et al.* [1940] showed that the secretion of the first part of the duodenum (which contains much mucus from Brunner's glands) was activated by vagal stimulation. It therefore seemed likely that the secretion of the intervening segment, the pyloric antrum, was influenced by the vagus, but this had never been clearly demonstrated. Experiments have now been done to investigate this point, and at the same time data have been collected bearing on mucus secretion from the fundus and cardia of the stomach. Cats have been used for all experiments.

Vagal Stimulation.

A. *Acute Preparations.*—In order to study the effect on pyloric secretion of stimulating the peripheral ends of the vagi, it was necessary to divide the pyloric antrum from the rest of the stomach, while avoiding damage to the nerves as far as possible. The best preparation we have used was made as follows. Under ether anæsthesia the pyloric end of the stomach was located through a mid-line abdominal incision. A cut was made with fine scissors through all layers of the stomach wall close to the greater curve starting about 3 cm. above the pylorus and extending for 2 cm. towards the fundus. A curved intestinal needle was put into the mucosa from the inside of the stomach at a point on the lesser curve opposite the lower end of the incision (about $2\frac{1}{2}$ cm. from the pylorus). An artery clip was attached to the pyloric end of the cut on the greater curve. By pulling on the needle and the artery clip two folds of mucosa at the junction of the pyloric antrum with the body of the stomach were brought into apposition. These folds were

[1] Holding a personal grant from the Medical R[illegible]

In control cats set up with a pyloric pouch but with no interference in the chest the pyloric antrum and fundus both slowly secreted a very mucoid fluid; one cat in 10¼ hours secreted 4·4 c.c. of pyloric juice and 2·3 c.c. of fundal juice, a second in 10½ hours 3·6 c.c. and 1·3 c.c. respectively, and a third in 12 hours 4·2 c.c. and 2·2 c.c. A similar preparation, but decerebrate instead of decapitate, secreted 10·2 c.c. of fundal juice and 3·0 c.c. of pyloric juice in 12 hours. A cat with the splanchnics cut in the chest secreted 7 c.c. of fundal juice and 2·5 c.c. of pyloric juice in 9 hours. Atropine did not consistently alter this "spontaneous" secretion. One decapitate cat produced 88 c.c. of watery fundal juice and 1·5 c.c. of mucoid pyloric juice in 11 hours after receiving 0·6 c.c. of 1 per cent. atropine intravenously followed 4 hours later by 1·0 c.c. In another animal 22 c.c. of very mucoid fundal juice and 8 c.c. of pyloric juice—the last part not mucoid—were collected in 9 hours. In a third cat 3 c.c. of fundal juice and 2 c.c. of pyloric juice, both very sticky, appeared in 11½ hours.

These experiments made it clear that this type of preparation produced a certain amount of typical pyloric secretion and a sticky mucoid very slightly acid fundal secretion in the absence of any direct nerve stimulation except such peripheral stimulation as might be associated with the trauma of the operation.

Stimulation Experiments.—Faradic stimuli (about 30 per second), 15 seconds on and then 15 seconds off, were applied to the vagi in the chest for long periods. In three experiments on transfused animals a copious fundal secretion gave evidence of effective vagal stimulation. One cat in 10 hours secreted 151 c.c. of fundal juice containing a considerable amount of "dissolved" mucus and 4 c.c. of alkaline very mucoid pyloric juice, the second in 9½ hours gave 210 c.c. of fundal juice and 6 c.c. of pyloric juice, and the third in 14½ hours 205 c.c. of fundal juice and 5·7 c.c. of pyloric juice.

The amounts of pyloric juice in these three experiments are of the same order as those in the unstimulated controls. The histological picture, however, is very different, and shows that vagal stimulation promotes a definite emptying of the pyloric gland cells which is not seen in the controls.

Histology.—Mucosa was taken at the beginning and end of all experiments and was placed in Helly's fluid. Bensley [1898] noted that an eosin-indulin-aurantia mixture stained the pyloric gland cells and certain cells of the fundal glands in mammals. The stain has been modified by Mr. H. Axtell in this laboratory as follows: indulin 2·0 g. is ground in 30 c.c. glycerol and the solution is diluted with 20 c.c. glycerol, 30 c.c. distilled water and 50 c.c. alcohol. Staining is done thus: (1) After removal of wax and any mercury deposit the section is thoroughly dehydrated with alcohol, (2) placed in picric-alcohol for 5 minutes, (3) transferred to indulin solution for 1–12 hours, (4) washed

sewn together, care being taken to include only mucosa and the minimum amount of submucosa; the cut along the greater curve was then closed in two layers. In this way the pyloric antrum was divided from the fundus with the least possible interference with its nerve supply. The pyloric pouch was cannulated through a cut in the duodenum 3 or 4 mm. below the pyloric ring, the ligature being placed so as to interfere with the blood-supply of the pouch as little as possible. A cannula was also tied into an incision in the fundus. Both cannulæ were brought to the outside of the abdomen through suitably placed stab wounds and the mid-line incision was closed. The vagi were dissected in the chest and an electrode applied for stimulation (see note at end). The animal was decapitated.

In some of the earlier experiments the condition of the cat, although it had appeared good at the end of the operation, rapidly deteriorated, so a therapeutic measure which proved to be of great value was adopted. Twenty c.c. of citrated blood from another cat were injected intravenously as soon as the operation was over and plasma was given at *suitable intervals afterwards; in other words, surgical shock* was combated. After the adoption of this procedure the cat remained in good condition even if the period of stimulation was extended to 10 or 12 hours. At the end of the experiment the animal was usually bled into citrate solution and the blood used for the next experiment.

After great loss of acid gastric juice (in vagal stimulation experiments) muscular twitching and movements occurred on the slightest touch, presumably because of alkalosis. In the last few experiments therefore the fundal juice, diluted with an equal quantity of water, was run slowly into the duodenum through a cannula, so that it could be reabsorbed from the small intestine.

Pyloric Juice.—From controls, vagal stimulation and permanent fistulæ alike the pyloric secretion was a sticky, clear, egg-white-like juice capable of neutralising from 0·0 to 0·2 c.c. of N/10 HCl per c.c. Blood pigment mixed with the juice was noticed to become a bright pink or lilac colour on standing. The juice from permanent fistulæ uniformly contained a trace of proteolytic enzyme active against albumen or casein at pH 2, but was totally inactive against these proteins at pH 6·8 and pH 8.

Fundal Juice from vagal stimulation was equivalent to 1·3 to 1·6 c.c. of N/10 HCl per c.c., and this high acidity was maintained throughout a period of stimulation lasting as long as 14 hours. When the pylorus was perfused with acid without nerve stimulation the fundus secreted a quite different juice—a scanty, egg-white-like material which clung to the mucosa and had a titration value of only 0·4 c.c. N/10 HCl per c.c. A fundal juice of similar appearance was secreted in the control animals.

experiments, but since the abdomen was not opened the stomach was completely untouched during the operation and there was therefore no local trauma. The animal was decapitated. The secretion was collected by applying a small test-tube over the mouth of the fistula. At the start of stimulation vigorous peristalsis of the pyloric segment could be seen owing to the attachment of the segment to the skin. After about half an hour the peristalis disappeared, but secretion continued at the same rate as before.

Protocols. Cat 1.—Pyloric fistula in good condition, inserted $6\frac{1}{2}$ months before. Cat starved for 24 hours; no secretion could be collected in the 2 hours before the experiment.

11.10–12.20	Preparation of vagi and decapitation.
13.00	20 c.c. blood intravenously.
12.20–14.20	*Control Period,* no stimulation. A small drop of juice collected at the mouth of the fistula.
14.25–16.25	*Stimulation.*—0·6 c.c. very mucoid juice collected in 2 hours.
16.25–18.25	*Control Period.*—0·2 c.c. juice collected in the first $\frac{1}{2}$ hour after stopping stimulation, nothing in the last $1\frac{1}{2}$ hours.
18.45–21.45	*Stimulation.*—0·45 c.c. very sticky secretion in 3 hours.
21.45–23·45	*Stimulation.*—0·20 c.c. very sticky secretion in 2 hours.
22.00	12.00 c.c. plasma intravenously.
23.45	Cat killed. The fistula was about 2 cm. long and in perfect condition. Histological examination showed a completely healthy pyloric mucosa.

Cat 2.—Pyloric fistula in good condition, inserted $2\frac{1}{2}$ months before. Cat starved for 24 hours; no secretion could be collected in the hour before the experiment.

10.50–12.15	Preparation of vagi and decapitation.
12.20–14.05	*Control Period,* no stimulation. No secretion.
14.05–16.40	*Stimulation.*—0·5 c.c. very sticky juice collected in 2 hours 35 minutes.
16.40–18.40	*Stimulation.*—0·75 c.c. juice in 2 hours.
18.40–21.40	*Control Period.*—One drop of juice at mouth of fistula, nothing in collecting tube in 3 hours.
18.50	15 c.c. plasma intravenously.
21.40–23.40	*Stimulation.*—0·35 c.c. very sticky juice in 2 hours.
23.40	Cat killed. Fistula about 2·5 cm. long and in good order. Histology—completely healthy pyloric mucosa.

Together with the histological picture seen after other acute experiments, these two protocols are strong evidence that the vagus is a secretory nerve to the pyloric glands.

quickly in water, (5) placed in 50 per cent. H_2SO_4 for 10 minutes and finally (6) washed well in water. As a routine it is then stained with mucicarmine and counter-stained with metanyl yellow.

The cardiac gland cells, the mucoid cells of the fundus, and the pyloric gland cells stain an intense greenish black, while the surface mucous cells are stained red by the mucicarmine. The nuclei are brown and the rest of the tissue various shades of yellow. Very precise pictures are obtained of the distribution of the mucin in the pyloric glands and information is available which cannot be obtained by any other method. The mucin of Brunner's glands stains in exactly the same way.

Figs. 3 and 4 illustrate that prolonged vagal stimulation causes almost complete evacuation of mucus from the pyloric gland cells. The lumina of the evacuated glands appear to be somewhat dilated. In some animals dark-staining mucus lies free in the dilated lumen. Under higher magnification it is possible to see a rim of mucin remaining at the free borders of the cells and dark-staining mucin granules in the Golgi body area—a phenomenon noted in other mammalian mucous cells after exhaustion [Florey, 1932; Florey and Harding, 1933]. The surface mucous cells, staining red with mucicarmine, display no evidence of secretory activity after vagal stimulation.

B. *Fistula Experiments.*—It was thought that the stimulus of trauma might be responsible for the secretion appearing from the pylorus in the "acute" preparation. To overcome this four innervated pyloric fistulæ were prepared. The operation was the same as in the acute preparation as far as the stage of holding up the two folds of mucosa with the curved needle and artery forceps. Instead of sewing these two folds together, each fold of mucosa was split with fine scissors so as to divide the pyloric antrum from the rest of the stomach; great care was taken to cut nothing but the mucosa and the least possible amount of submucosa. The pyloric and fundal ends of the mucosa were each insewn and the split in the muscle near the greater curve was closed. The pyloric ring was cut across and the stump of the duodenum infolded. A posterior gastro-enterostomy was made. The mouth of the pyloric pouch was stitched into a conveniently placed stab wound in the belly wall. When the fistula had healed and the feeding experiments to be described later had been carried out, the vagus was stimulated in a terminal acute experiment.

In two of the four cats the acute experiments unfortunately failed. One animal died from under-ventilation during preparation, and in the other the electrode gave trouble so that stimulation was ineffective except at the beginning of the experiment, when some secretion appeared. The remaining two cats, however, yielded convincing evidence that stimulation of the vagi in the chest caused secretion to appear in the pyloric pouch. The vagi were prepared as in other acute

3 hours 20 minutes in one cat and 6 hours 20 minutes in another produced only a very small output of fluid (1·2 c.c. and 1·3 c.c. respectively) and no significant neutralisation. Histological investigation showed that the pyloric mucosa had not been damaged by the fundal juice, neither was there any evidence that either the surface or the gland cells had been activated. A few c.c. of thick egg-white-like mucus collected in the fundal pouch during the perfusion.

In six experiments done in a similiar way the vagi were stimulated during the perfusion of fundal juice or of HCl of equivalent acidity. Very slight neutralisation occurred, but there was no appreciable increase in the stickiness of the perfusate. In some experiments there was a small absorption of fluid. Histologically, activity of the pyloric gland cells was shown by the partial or complete evacuation of mucus into the gland lumina, which were dilated. This evidence of activity was never seen except after vagal stimulation. The surface epithelial cells were inactive.

The results of these experiments contrast with those obtained on the duodenum by similar methods. The duodenal mucosa is considerably damaged by perfusion with N/10 or stronger HCl, while the pylorus is unaffected by N/6·6 HCl or gastric juice of about the same strength. Moreover, the mere perfusion of acid does not appear to stimulate the mucus-producing cells in the pyloric antrum, whereas Brunner's glands are activated by this procedure even in a heavily atropinised animal.

Mucus Secretion in the Fundus.

There are two possible sources of mucin in the fundal mucosa—the surface epithelial cells and the mucoid cells of the glands, which are chiefly clustered in the neck region though a few are present in the depths of the glandular crypts. By using Axtell's stain it has been possible to pick out the mucoid cells quite certainly and distinctly and to examine the effect of prolonged vagal stimulation on their secretion. Control pieces of fundal mucosa were taken at the beginning of each experiment. As well as those experiments in which the pylorus was divided from the fundus, two were done in which the only interference with the stomach was the insertion of a cannula into the fundus and the placing of a ligature just distal to the pylorus.

In all vagal stimulation experiments there was a very severe depletion of the contents of the peptic cells, but in none was complete exhaustion of the mucoid (dark staining) cells observed. Some cats, even if they had secreted large quantities of mucoid fundal juice, showed no certain difference between the mucoid cells of the control and stimulated mucosa. In others, however, although many mucoid cells retained a considerable amount of mucin, dark-staining mucin was found in the gland lumina after stimulation, a phenomenon never seen in control

Feeding Experiments.

Other workers, using dogs [*e.g.* Ivy and Oyama, 1921], have found that pyloric pouches, whether innervated or denervated, secrete continuously without regard to feeding or starvation. In some previously unpublished experiments [Florey and Harding, 1934] this has been confirmed for denervated pouches. One pouch, for example, completely severed from the fundus so that all vagal fibres in the muscle were cut, regularly secreted 0·2 c.c. of thick mucoid juice in an hour whether the animal was fed or not. Another pouch, totally transplanted into the subcutaneous tissues by the same method as had been used for the duodenum [Florey and Harding, 1935] and therefore deprived of all vagal and sympathetic fibres, secreted the typical mucoid alkaline juice at the rate of 0·15 c.c. an hour, again without regard to food. This fistula preserved its structure excellently, the gland cells being particularly full of mucus when it was seen histologically, and it showed no evidence of inflammation.

We have now obtained definite evidence that pyloric pouches, if fully innervated, can react to feeding and starvation. The four permanent pouches in the series of cats which gave clear results in terminal vagal stimulation experiments were prepared, so that the whole nerve supply was carefully preserved. The fistula healed perfectly and the cats remained healthy throughout the experiment—one for 6½ months. The fistulæ secreted the typical mucoid pyloric juice like egg-white, and in three out of the four cats gave a well-marked reaction to feeding. After 24 hours' starvation the mucosa at the mouth of the fistula was moist, but no juice could be collected in a pot tied over the opening. If meat and milk were then fed secretion began immediately and juice collected at the rate of 0·2–0·3 c.c. an hour. This was repeatedly confirmed in three cats. The fourth cat gave no such effect, rather food seemed sometimes to inhibit the secretion, which was maximal 4–5 hours after feeding; no explanation for this variation was forthcoming.

Perfusion.

It was noted by Florey and Harding [1933] that perfusion of dilute HCl through the first part of the duodenum activated Brunner's glands and caused partial neutralisation of the perfusing fluid. This phenomenon has been confirmed by us many times since the original observations. Similar experiments have now been performed on the pylorus by perfusing HCl or gastric juice through pyloric segments prepared as described earlier in this paper, but with the addition of a cannula in the proximal end of the pouch for inlet of the fluid. The perfusate was warmed to 37° C. by a water-jacket around the entry tube, the temperature being kept constant by a thermostat.

The perfusion of fundal juice at the rate of 10–12 c.c. an hour for

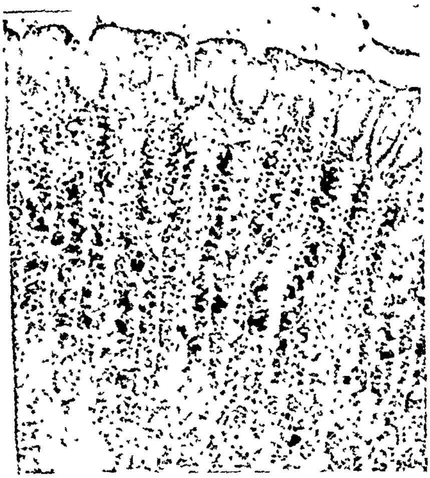

FIG. 1.

FIG. 2.

FIG. 1.—Normal fundal mucosa taken before stimulation. Axtell's stain. The mucoid cells appear black and the peptic cells grey. × 75.

FIG. 2.—Fundal mucosa from same cat as fig. 1 after 6 hours of vagal stimulation. Note that the mucoid cells have been depleted and that the peptic cells appear exhausted. × 75.

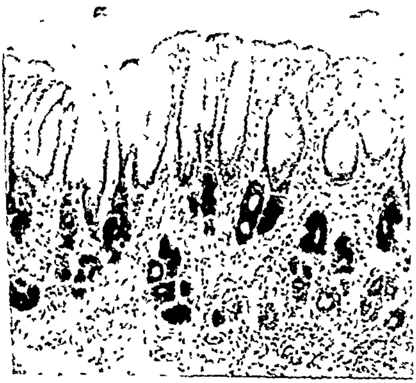

FIG. 3.

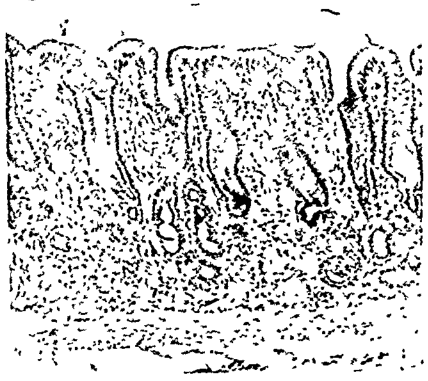

FIG. 4.

FIG. 3.—Control piece of pyloric mucosa taken at beginning of experiment. Axtell's stain. Mucus in the cells of pyloric glands appear black. × 75.

FIG. 4.—Pyloric mucosa of same cat after $10\frac{1}{2}$ hours of vagal stimulation. Note the almost complete disappearance of dark-staining mucus. A thin rim of mucus at the free borders of the cells can be seen in some glands. × 75.

JENNINGS and FLOREY, "The Influence of Vagus on Secretion of Mucus by the Stomach."

sections. As in the pylorus, the surface epithelium showed no activity. Figs. 1 and 2 illustrate the most marked exhaustion of the mucoid cells which was seen.

The fundal juice obtained in all these experiments was mucoid, in some cases particularly so, and the mucin, which was intimately mixed with the juice, did not separate out on standing. In addition to the dissolved mucin separate strands of thick mucin were frequently present. This mucoid juice contrasts sharply with that produced by histamine. Of two cats, one received 5·5 mg. of histamine in divided doses and secreted 74 c.c. of juice from the whole stomach in 6½ hours, and the other received 4·75 mg. and secreted 62 c.c. in 6 hours. The juice was just as acid as vagal juice, but differed from it in looking like water and having apparently *no mucin content. Histological* examination disclosed no changes in the mucous cells.

Cardiac Glands.—These glands lie just distal to the œsophageal junction, and in the cat they are small. The mucin contained in the cells stains with indulin in the same way as in the mucoid and pyloric *cells. In all experiments with prolonged vagal stimulation the cells* were depleted or exhausted of their mucin.

Discussion.

Babkin in 1928 reviewed the work done up to that time on the innervation of the pyloric glands and noted that the results were few and *contradictory. Since then, in Babkin's laboratory,* Vineberg [1931] has studied the secretion of mucus by the stomach and his results have been confirmed by Baxter [1934]. Vineberg was concerned with the secretion of the stomach as a whole rather than with that of the pylorus in particular, but his results agree with those described in this paper, *i.e. that vagal stimulation causes secretion* of mucus by the pyloric glands. Gitlitz and Levison [1936] showed in rabbits that injection of pilocarpine emptied the pyloric antral cells of their mucus, and described changes in the Golgi apparatus accompanying the discharge and subsequent fresh production of mucus.

Vineberg demonstrated also that slow weak rhythmic stimulation of the vagi in the neck of dogs and cats produced a mucous secretion which he believed to come from the mucoid cells of the fundal glands (the indulin-staining cells of the present work), but he recorded no histological observations. Stimulating the vagi faradically (30 per second) we have always obtained a copious *acid mucoid secretion from the* fundus, and often histological evidence of depletion of the mucoid cells, though the evacuation is never so complete as in the pyloric gland cells. The War has, unfortunately, prevented further experiments using the slow stimuli advocated by Vineberg, which perhaps might give a more complete exhaustion.

The cardiac glands have shown well-marked histological exhaustion in all our vagal stimulation experiments.

The evidence therefore is that the vagus nerve controls the secretion of the cardiac mucous glands, the mucoid cells of the fundus and body of the stomach, the pyloric glands, and the mucous cells of Brunner's glands in the duodenum [Wright *et al.*, 1940]. In some experiments on man with atropine Loeper and Fau [1933] reached the opposite conclusion, *i.e.* that the vagus inhibited the production of mucus in the stomach, but their work is slight and would need confirmation.

Baxter [1934] has concluded that the sympathetic nerves also have a secretory influence on the mucous cells of the stomach, but it is worth while to call attention to the fact that in unanæsthetised decapitate cats considerable amounts of mucus are secreted in the pylorus without any specific nerve stimulation, and that even permanent denervated fistulæ may secrete spontaneously. Histological confirmation of increased activity under sympathetic nerve stimulation is desirable.

A point of interest arising from our experiments is the strength of the HCl secreted in the fundal juice during vagal stimulation and the height at which the titre was maintained throughout many hours of stimulation.

Attention is drawn to the very beneficial effects of following up a complicated acute operation with a blood transfusion. The improved condition of preparations treated in this way is most striking. Blood transfusion with, if necessary, subsequent plasma transfusion may be useful in many physiological experiments.

Summary.

1. Evidence is produced that the vagus controls the secretion of the cardiac and pyloric mucous glands and the mucous neck cells of the fundal glands.
2. A histological procedure is described for staining these cells selectively and intensely.
3. No effect of vagal nerve stimulation on the surface epithelial cells of the stomach has been seen.
4. Innervated pyloric pouches cease to secrete on starvation, but secrete at once following the ingestion of food.
5. Blood transfusion has been found greatly to improve the general condition of the decapitate cat after a long and complicated preparation.

Note on the Electrode and its Application.

The electrode used in these experiments and in those on Brunner's glands [Wright *et al.*, 1940] was devised by R. D. Wright (fig. 5). It

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consists of a small rectangular block of Perspex in which an oval recess is cut. A hole is drilled through each end into the recess, large enough at one end to admit both vagi, smaller at the other end. Two platinum wires are fixed across the recess and are soldered to long pieces of thin enamelled wire. Before application a fine silk thread is passed through the holes and over one platinum wire and under the other in the recess, and a small piece of thick rubber (a chip from a rubber tube) is threaded by a needle on to the end emerging from the smaller hole. The recess is then made into a closed chamber by stretching tightly over it a piece of thin rubber, which is twisted and tied at the back of the electrode.

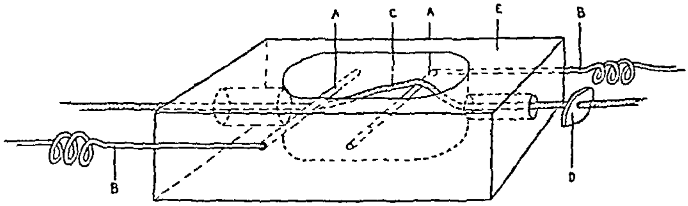

FIG. 5.—Outside measurements of block: 13 mm. long, 8 mm. wide, 3·5 mm. deep. Recess 7 × 4 mm. Junctions of platinum and enamelled wires are buried in perspex cement. AA, platinum wires; BB, enamelled wires; C, silk thread; D, piece of thick rubber; E, Perspex block. The covering of thin rubber is not shown.

The method of opening the chest has been previously described [Wright *et al.*, 1940]. In the present experiments the right and left vagi were dissected as high as the root of the lung, so that 2–3 cm. were freed. The posterior communicating branch was also dissected. The nerves were cut as near the root of the lung as possible and the right trunk was passed behind the œsophagus, so that all three nerves lay in the left para-œsophageal groove. The cut ends of the nerves were tied together with the free end of the silk thread and the nerves were gently pulled into the chamber, into contact with the platinum electrodes. The chip of rubber was slipped down the thread till it touched the Perspex block, gripping the thread and preventing the nerves from slipping out. In this way the electrode with the nerves in it could be made to lie conveniently behind the œsophagus without kinking.

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We are indebted to Mr. J. Kent for technical assistance.

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CONSTRICTION OF RENAL VESSELS IN THE FROG (*RANA ESCULENTA*) AND ITS EFFECTS ON THE SYSTEMIC BLOOD-PRESSURE. By MARTHE VOGT.[1] From the Pharmacology Laboratory, Cambridge.

(*Received for publication 7th November* 1940.)

THE experimental production of hypertension in mammals by chronic obstruction of the blood-flow to the kidney was first described by Goldblatt, Lynch, Hanzal, and Summerville [1934] and has since been generally confirmed. Work carried out in Goldblatt's and in other laboratories has shown that liberation by the kidneys of some chemical substance is to be considered as the cause of the hypertension. Nothing, however, is known about the site or mode of production of that substance in the kidney. Experiments described by Verney and Vogt [1938] have shown that the degree of hypertension increases with the functional load to which the kidney is subjected. This fact suggests a link between renal metabolism and production of the hypothetical agent. Since the tubules are supposed to have a larger share in the renal metabolism than the glomeruli, it seemed possible that ischæmia of the former was directly responsible for the production of the unknown substance. An attempt is described in this paper to test this deduction on frogs, since their double renal blood supply, the portal circulation feeding tubules (of the first order) only, and the arterial supply irrigating glomeruli and tubules of the second order, affords a means of producing an isolated ischæmia of tubules.

METHODS.

Eighteen specimens of *Rana esculenta* (Hungarian variety) were available for the experiments. In order to take repeated readings of their blood-pressure, the frogs were anæsthetised with urethane or ether and, as soon as their respiration had stopped, placed on a horizontal board adjacent to the stage of a microscope. The tongue was gently pulled out of the mouth, passed through a 1·5 cm. long cuff which could be inflated, and its forked tips were spread on a slide, moistened with water and covered with a coverslip. Observation of the lingual vessels was made with a low-power lens (Leitz No. 3).

The cuff was similar to that described by Verney and Vogt [1938,

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abdominal vein. The renal portal veins were exposed at the distal pole of the kidneys. For the reduction of renal arterial supply the aorta was constricted immediately below the origin of the cœliaco-mesenteric artery, thus producing an ischæmia of both kidneys simultaneously. The anastomoses described by Kempton [1937] between the posterior renal arteries on the one hand and the iliac and inferior mesenteric arteries on the other are included in the area of diminished circulation. The anæsthetics used for the operations were urethane or ether.

Results.

1. *Obstruction of the Renal Portal Veins.*

The preliminary ligation of the abdominal vein had no effect on the blood-pressure of eleven frogs, although three of these died suddenly a few days after the operation.

In five of the eight surviving frogs of this series, *one* portal vein *only* was obstructed. The blood-pressure remained unchanged in all of them during the period of observation, which lasted from three to twenty-one days, except for one frog in which the pressure fell below normal from the fifteenth post-operative day onwards. The efficacy of the constricting ligature is shown by a mild shrinkage of the kidney on the operated side which was observed in four of the frogs. The difference in length had reached 4 and 4·5 mm. in two frogs which were killed ten and twenty-one days respectively after the operation. Histologically, the only obvious difference was the larger percentage of dorsal tubules of a small diameter in the operated as compared with the normal kidney. The findings show some resemblance to the diminution in size seen in the ischæmic kidney of hypertensive dogs, but the effect on the blood-pressure is nevertheless absent.

In three frogs *both* portal veins were obstructed. The two sides were done simultaneously in one frog; this resulted in a fall of blood-pressure ending in death thirteen days later. Done in two stages, the operation was survived indefinitely (observation for two months, see F. 8 of fig. 1) in one frog, a transitory fall being produced by the second operation; and was survived for one day only in another (F. 11 of fig. 1), the blood-pressure remaining unaffected.

Obstruction of the renal portal vein, therefore, in the frog whose abdominal vein has previously been ligated, does not produce a rise in systemic blood-pressure.

2. *Obstruction of the Abdominal Aorta.*

The aorta was obstructed in seven frogs. The results are illustrated in F. 20 and F. 22 of fig. 1. No effect whatever on the blood-pressure was seen. The frogs were observed for periods up to thirty

p. 256]. Its width, however, was reduced to half, and only the outer strip was made of thick rubber. A piece of thin rubber formed its inner surface and its side walls, and was sealed to the outer surface of the thick strip. In making these cuffs, care has to be taken that the inner rubber is slack, so that very little pressure is needed to occlude the cylindrical channel through which the tongue is passed and in which it is compressed. This channel, which has a diameter of about 9 mm., is kept at constant size by a ligature tied round the free ends of the cuff.

The cuff was connected with an air reservoir whence one piece of tubing led to a mercury manometer and another to a compression ball. An arteriole was selected for observation, the cuff inflated till the pulsation in the artery stopped, and the pressure gradually released in order to find the point at which a pulsatile flow was resumed. This point corresponded with the systolic blood-pressure.

The accuracy of the cuff method was checked by recording the mean blood-pressure of a frog (the abdominal aorta was cannulated with a heparine-filled cannula connected to a narrow bore mercury manometer), and simultaneously observing the pressure as estimated by compression of the tongue. The cuff readings were found to be 4 mm. higher than the mean blood-pressure as recorded from the abdominal aorta.

Deep anæsthesia, though necessary for the purpose of pulling the tongue out of the frog's mouth, lowers the blood-pressure considerably. It is, however, possible to continue the observations during the whole period of the frog's recovery from the anæsthesia, since the tongue, once it has been pulled out, will not be withdrawn so powerfully as at the beginning of the anæsthesia. By taking readings up to the time when the frog comes round and starts to move, a maximal value is obtained which provides a reasonably steady base-line. Needless to say, the frogs are always breathing when the significant readings are taken. Measurements were usually repeated at intervals of 24 or 48 hours. The first readings on a new frog, or readings after a long pause in the observations, are often lower than normal, since some frogs start to withdraw their tongue from the cuff in a deeper stage of anæsthesia than after some days of "training."

The experiments were done between May and October on frogs of both sexes weighing between 47 and 96 g. and fed on earthworms and insects.

The renal vessels were constricted by tying a silk ligature tightly around a pin and the particular vessel, and subsequently removing the pin. If obstruction of the flow through the renal portal veins was intended, the abdominal vein was tied in a preliminary operation, since it was feared that the effect of the ligature might otherwise be a more or less complete shift of the circulation from the renal portal into the

absence of any rise from the combined procedure; if a change was produced at all by constricting the aorta, it was in the direction of a fall in blood-pressure.

Ischæmia of the kidneys produced by constriction of the aorta above the origin of the renal arteries, either by itself or combined with obstruction of the renal portal veins, has, therefore, no influence on the frog's blood-pressure.

Summary.

A method is described which permits daily readings of the frog's systemic blood-pressure to be made in light anæsthesia.

Constriction of one or of both renal portal veins was performed in frogs (*Rana esculenta*) in which the abdominal vein had been ligated. The constriction had no effect on the systemic blood-pressure.

Obstruction of the aorta just above the origin of the renal arteries in *Rana esculenta* did not alter the frogs' blood-pressure, neither did this operation do so in combination with constriction of the renal portal veins.

Some of these experiments were done in the Molteno Institute for Parasitology. It is a pleasure to thank Professor Keilin for the facilities he gave me.

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days. That the absence of effect could hardly be due to insufficient arterial constriction was demonstrated by (*a*) a fall in blood-pressure and death of the frog if the constriction was made only slightly severer than in the experiments in which the frogs survived, and (*b*) an atrophy of the kidneys seen at the post-mortem examination of the operated

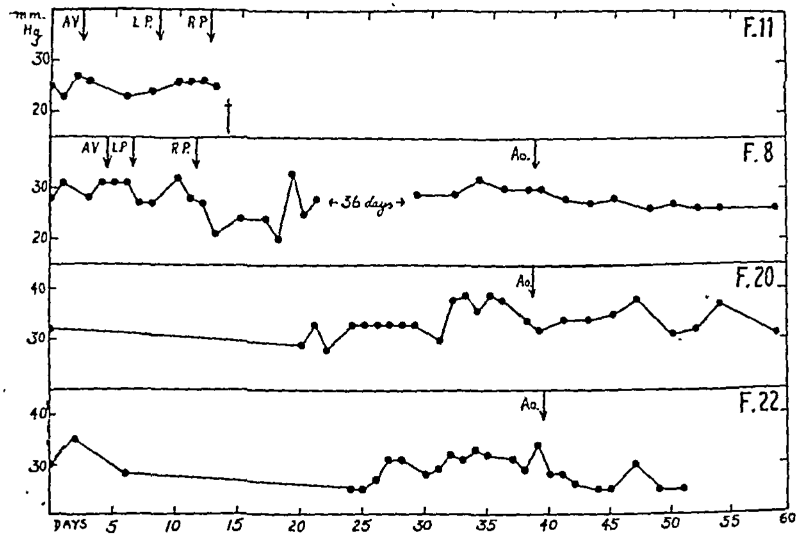

FIG. 1.

F. 11, ♂, 62 g. F. 20, ♂ 48 g.
F. 8, ♀, 82 g. F. 22, ♀, 55 g.
A.V. = Ligation of the abdominal vein.
L.P. = Constriction of the left renal portal vein.
R.P. = Constriction of the right renal portal vein.
Ao. = Constriction of the aorta just above the renal arteries.
Abscissa: *days*.
Ordinate: systolic blood-pressure in mm. Hg.

frogs. Since the obstruction was symmetrical, the degree of atrophy was difficult to assess, but the atrophy seemed too regular to be accidental, and consisted in a thinning of the organ in the dorso-ventral direction. Histologically one frog only (No. 20) had serious renal lesions: when killed twenty-six days after the operation, acidophilic fluid was found in the capsules of nearly all glomeruli and in many tubules of the first order. In the other frogs nothing abnormal was seen with the exception of some atrophied glomeruli and casts in a few dorsal tubules of two of them.

3. *Constriction of the Renal Portal Veins and of the Aorta.*

In F. 8 constriction of the aorta was carried out when complete recovery of the blood-pressure from its transitory fall after the operation on the second vein had taken place. The graph (see fig. 1) shows the

HUNGER MOTILITY IN A HYPNOTISED SUBJECT.[1] By

R. E. SCANTLEBURY and T. L. PATTERSON. From the Department of Physiology, Wayne University College of Medicine, Detroit, Michigan.

(*Received for publication 2nd September* 1940.)

IN a study of psychophysiology the gastric mechanism during hunger is an ideal starting-point. It is subject to marked changes due to psychic stimuli and records of these changes may be readily made.

THE EXPERIMENTAL PROCEDURE FOR THE STUDY OF PSYCHIC PHENOMENA ON THE GASTRIC HUNGER MOVEMENTS.

In our experiments the balloon method of recording was used. The balloon was introduced by the œsophageal route and the connecting tube was attached to a bromoform (sp. g. 2·884) manometer. A single-drum smoked paper kymograph was used in the first recordings, followed by a longer series made on a long-paper Harvard kymograph revolving at the same speed. The ink method of recording was used in the latter series [Patterson, Scantlebury, and Gijsbers, 1935; Patterson, 1937, 1938]. This provided sufficient recording surface for an entire afternoon's work (fig. 1).

Three subjects, R. E. S., H. L. F., and N. G., were used for a series of normal and experimental studies totalling about one hundred and fifty hours. These subjects fasted for about eighteen hours preceding recordings, and the experimental period lasted from four to six and a half hours, the total fast period being twenty-two to twenty-four and a half hours.

The normal hunger periods of our subjects fell within the average for the twenty-six- to thirty-four-year age-group [Carlson, 1912]. A constant twenty to thirty seconds' tonus rhythm was maintained throughout the series, and where definite inhibitory influence did not enter into the contraction period the incomplete tetanus ending was usually present. A total of sixty hours of tracings was taken from the stomach of R. E. S., seventeen hours of which were normal and

[1] Preliminary reports of this work were given before the American Physiological Society at Detroit, Michigan, 11th April 1935, and at Baltimore, Maryland, 31st March 1938, brief abstracts of which were published in the *Proceedings* of that Society (*Amer. J. Physiol.*, 1935, **113**, 47; *ibid.*, 1938, **123**, 179).

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forty-three hours experimental recordings. The tracings used from the work on H. L. F. consisted of a total of fifty-two hours, twelve hours of which were normal recordings, with psychic and hypnotic

FIG. 1.—Photograph showing the arrangement of apparatus for recording gastric movements. The tracing is of a normal hunger period.

variations being used during the remaining forty hours. The normal studies from the stomach of N. G. varied so greatly and were so limited in number that the experimental results were not considered.

The Effect of Normal Sleep on the Hunger Contractions.

In order to reach a fair estimation of the effect of hypnotic and other psychic influences on gastric motility it seemed advisable to study first the effect of normal sleep on hunger peristalsis. Carlson [1916] pointed out that during sleep there is a decreased activity of all neuromuscular mechanisms so far investigated, and further states that one might have expected that gastric tonus would have been likewise diminished. However, instead of being depressed, the hunger contractions continued with the same vigour as during the waking state.

Our subjects, as those of Wada [1922], when awake at the onset of the contraction period, expressed a feeling of extreme discomfort. This restlessness disappeared within five to ten minutes after the contractions commenced, when the subjects frequently fell asleep. Actual measurements of tracings showed that the height of the individual contractions were, on the average, greater during sleep. Too much emphasis should not be placed on this observation, however, since

the subjects more often slept during the period of vigorous contractions. It does confirm, however, that the contractions are not diminished during sleep.

The Effect of Hypnotising the Subject on Gastric Motility.

In the accompanying tracing (fig. 2) hypnotism was in progress between points 1 and 2. At point 2 the subject, H. L. F., was in a satisfactory state of hypotaxic hypnotism and suggestion was discontinued. Hypnotic procedure seemed in all cases to lessen the height of the individual contractions. The contractions returned to *normal* height when the subject was in a satisfactory hypnotic sleep.

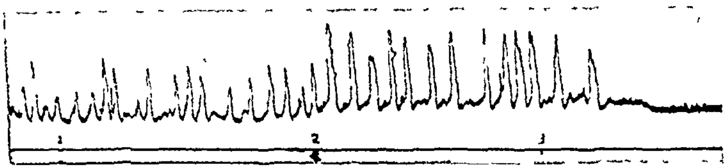

Fig. 2.—The effect of producing hypnosis is shown between points 1 and 2. At 2 the subject was in satisfactory hypotaxic hypnotic sleep. Point 3 denotes the introduction of the "hypothetical meal."

We concluded that the production of hypnotic sleep has a depressing effect on gastric motility. In most of our experiments, therefore, we placed the subject in hypnosis during the quiescent period. No effect on the tonus rhythm was noted, and only a slightly elevated general tonus was observed from the procedure in the period of quiescence.

The Effect of Psychic Stimuli on the Hunger Movements of the Waking Subject.

1. *The Sight and Smell of Food.*—The effect of the sight of food on the hunger contractions of man cannot be predicted. Carlson [1916] found, when acting as a subject himself, after fasting four days, that the sight of food completely inhibited his hunger contractions. Wada [1922] showed his subjects food and allowed them to smell it without obtaining any inhibition of the hunger movements.

In our investigation we sought to determine, if possible, which of these views was correct. In our subject, R. E. S., we did not find that the sight or smell of food had any effect on the hunger movements. A slight salivation was noted. It is significant that Carlson reported considerable salivation, while Wada said nothing about it. It would seem evident from our findings as correlated with those of other investigators that the amount of inhibition from the sight or smell of food depends largely upon the individual response to the stimuli. If the production of appetite juice or the cephalic phase of gastric secretion is sufficiently great there is evidently an inhibition.

2. *The Taste of Food.*—Our data from man concerning the effect of the taste of food verifies the work of Carlson [1916] and Wada [1922]. The taste of food inhibited the hunger contractions in subject R. E. S., who was given small bits of banana and on other occasions bits of chocolate cup-cakes. The degree of inhibition depended upon the strength of the stimulus. We found that the taste of food was always sufficient to produce some inhibitory effect on the hunger mechanism.

The Effect of Psychic Influence on the Hypnotised Subject.

In our series of more than eighty hours of recordings with the subjects in hypotaxic hypnosis, we suggested the eating of a standard

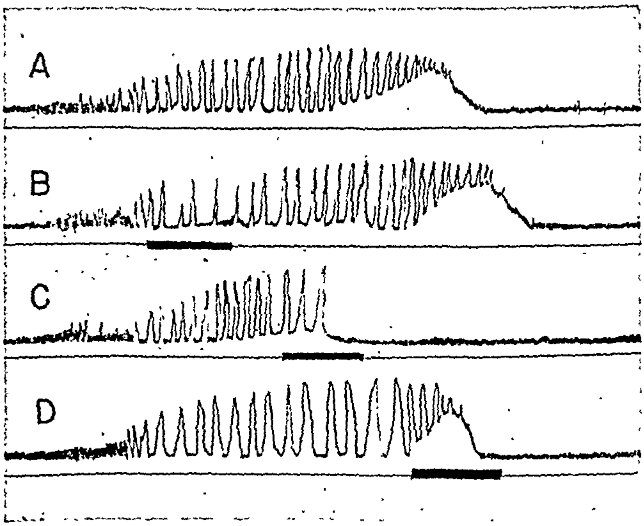

Fig. 3.—Composite tracing showing the result of introducing stimuli with respect to the length of a normal period. A, a normal contraction period thirty-one minutes in length. B, "hypothetical meal" given during first two-fifths of the normal period. C, same stimulus during second two-fifths of the normal period. D, same stimulus in last one-fifth of the normal period (period of incomplete tetanus).

hypnotic meal and compared the results with the normal influence of thought, sight, and smell of food. Our standard stimulus to the subject was the suggestion of eating a meal of soup, roast goose, sweet potatoes, peas, salad, and dessert. When the "hypothetical meal" was given during the period of gastric activity there was always some inhibition, with the exception of the tetany period.

We discovered variations in our results which depended upon the relative place in the period of activity at which the stimulus was given. Studies of our tracings were made, and we found that the type of reaction depended upon the relationship of the point of stimulation to the average length of the normal contraction period of that subject

With this hypothesis we arbitrarily divided our points of stimulation into three periods. Fig. 3 represents the composite effect of stimuli in the various periods as follows. A is a normal, B the first two-fifths of the normal contraction period, C the second two-fifths of the normal period, and D the final one-fifth or period of incomplete tetanus.

When the stimulus was given during the first one-fifth of the normal contraction period there was a temporary inhibition of the hunger movements and, as is indicated in fig. 4, B, the total length of the contraction period was greater than that of the normal (fig. 4, A). Fig. 4, B, from the stomach of R. E. S., shows results obtained from stimulation during the first one-fifth of the normal period. Suggestion of the

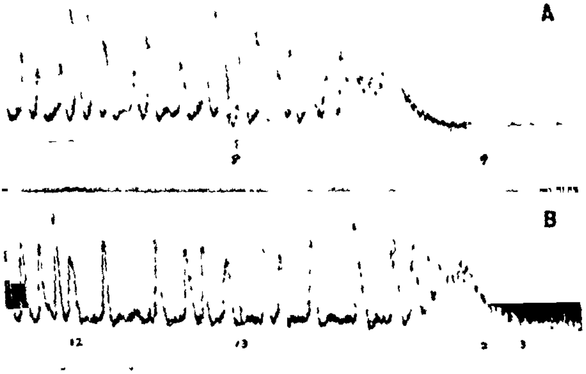

FIG. 4.—Subject R. E. S. A, normal hunger contractions ending in incomplete tetanus; 8, bodily movements; 9, subject snoring lightly. B, tracing showing the r[illegible] gestion in early part of contraction period; 12 to 13, the sugg[illegible] hypothetical meal." Note inhibitory effects. 2 to 3, hunger was without effect. Time one per second.

hypothetical meal commenced at point 12 and ended at point 13. Note the lengthening of the period between contractions [Scantlebury and Patterson, 1938]. The normal rhythm returned just at the end of the period and the incomplete tetanus ending is shown. The total length of this period was thirty-eight minutes, although the average for this subject was thirty and a half minutes, indicating that the period was sufficiently longer to compensate for the temporary inhibition.

The tracings in fig. 5 show the results of suggestion of the standard meal eighteen minutes after the appearance of the first contraction, during the second two-fifths period as indicated by fig. 3, C. In tracing A, fig. 5, the stimulus was commenced at point 2 and stopped at point 3. In tracing B, fig. 5, stimulus started at point 9 and ended at point 10. Note the complete inhibition following a short latent period of two to four minutes. These factors were characteristic of our records when the stimulus was introduced during the second period.

Fig. 6 indicates the effect of a stimulus introduced during the period

of incomplete tetanus, the third period as indicated in fig. 3, D. Carlson [1916] states that, when given during the period of incomplete tetanus, a sufficiently strong stimulus will give rise to an inhibition. We were

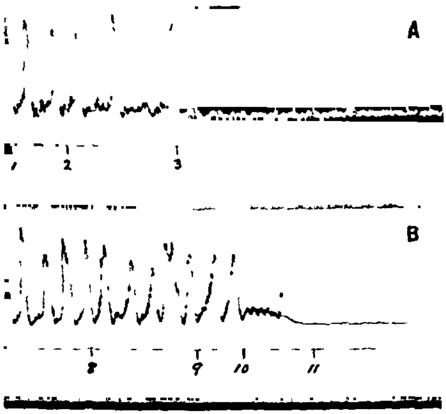

FIG. 5.—Subject R. E. S. A, 1, conclusion of hypnosis; 2 to 3, suggestion of eating a "hypothetical meal." Note complete inhibition. B, 8, suggestion of anxiety; 9 to 10, suggestion of eating a "hypnotic meal." Note complete inhibition. 11, subject still in hypnotic sleep. Both stimuli were given during the second two-fifths of the normal period length. Time one per second.

FIG. 6.—Results of the "hypothetical meal" given during the last one-fifth (period of incomplete tetanus) of the normal period length. 1 to 2, stimulus introduced. Note absence of inhibition.

unable to confirm these results (fig. 6). Our stimulus may not have been strong enough, or the period from the beginning of the stimulus to the normal ending may have been too short for the normal reflex to be completed.

RESULTS AND DISCUSSION.

An outline of the phenomenon of the state of immobility, akinesia, or mechanohypnosis, as it has been shown to exist in various animals through the phylogenetic scale, is shown in the table [Rabaud, 1918; Reisinger, 1926; Hoagland, 1928; Bleich, 1928; Bonnet and Saboul, 1935; Mangold and Eckstein, 1919; Steiniger, 1937; Spiegel and

Goldbloom, 1925; Ingram, Barris, and Ranson, 1936; *et al.*]. It will be noted that the condition of thanatosis as it occurs in nature, as well as the artificially produced state of akinesia, occurs in most of the lower forms studied. In the "feigned death" as it occurs in nature, the duration of the immobilisation is short. The akinesia produced in animals has been found by other investigators to last from 1 to 14 minutes; our preliminary studies in one series indicate that a state may be produced which will last up to 1 hour and 50 minutes in the frog. The seemingly significant factor in determining the duration of the period was the strength and length of the stimulus and the occlusion of air currents from the animal. It seems reasonable to assume that the sum total of the central nervous system involvement in such a state is purely due to the failure of a normal number of afferent stimuli to reach the brain, or a suppression of the pathway of the efferent stimuli passing the thoracic ganglia.

In the intermediate vertebrates (reptilia, aves, etc.), central nervous function may be partially depressed due to oxygen lack. This phenomenon certainly plays a part in the animals above the first transitional zone (see table), in which the vasomotor mechanism plays a more important rôle. In this group a long-continued change of position which affects the pressure in the carotid sinus mechanism produces a state termed mechanohypnosis. Either failure of the venous drainage or over-oxygenation of the vital centres will produce a typical catalyptic rigidity.

The term animal hypnosis has been applied to a state produced in the dog by Pavlov [1922]. A thorough examination of Pavlov's published data must place this state in the classification of immobilisation and not true hypnosis. In true hypnosis as it is found in man there is the condition of rapport, a mental contact between operator and subject. It is true that Pavlov produced a state in dogs in which they would respond to certain stimuli when they were under a state of immobilisation. The reverse was also true; that is, the stimulus would produce the immobilisation state. It seems then that the state so produced was really a conditioned reflex.

Differing from the conditioned response produced in dogs by Pavlov, the state of rapport is so established in man that during a single trance several suggestions not previously used by the operator may be acted upon by the hypnotised subject.

Boldyreff [1914] has reported that the contractions of the empty stomach are inhibited during periods of spontaneous secretion of gastric juice. Questions of the mechanism involved in this inhibitory action arise. Is the mechanism a direct stimulation of the stomach mucosa due to the process of secretion? Is it due to reflex action?

Pavlov [1910] from his work on dogs found that there is a latent period of about five minutes from the beginning of secretion to

Classification of Zones and Types of Hypnotic Response.

Invertebrata	Arthropoda Mollusca		Thanatosis	Occurs in nature.
Vertebrata	Amphibia Reptilia Aves		Akinesia	Immobilisation, mobilisation reflex due to the stimulation of sensitive zones (wing roots, thoracic ganglia, etc.). Lasts from 1 to 14 minutes.
			Transitional zone	Due either to stimulation of sensory skin zones (akinesia) or to vasomotor changes from carotid sinus afferent impulses. Anoxemia of certain motor areas due to changes in the O_2 content of the blood may be an important factor.
	Mammalia	Mouse Guinea-pig Rabbit Cat Dog	Mechanohypnosis or Animal hypnosis (?)	A spread or irradiation of inhibitory impulses over numerous motor areas of the cortex which produces a condition resembling partial sleep. In certain animals there may be a conditioning of the reflex responses which resemble true hypnosis.
		Chimpanzee	Transitional zone (?)	
		Man	True hypnosis	When the hypnotic state is produced in man by eliminating all external stimuli by causing an irradiation of inhibitory impulses over the motor cortex. A single avenue of approach is left open by means of positive suggestion through which a condition of rapport is established. In man hypnotism is of central origin, while in animals it is dependent upon some type of afferent stimulation.

inhibition. Carlson [1916] has set the latent period from "psychic" secretion in man at two to three minutes. It has also been shown that acid placed directly in the stomach causes an inhibition of the contractions; the amount and duration of alteration depends upon the total amount of hydrochloric acid introduced. The duration of inhibition is on the whole proportional to the total quantity of acid introduced.

It seems certain that the quantity of free hydrochloric acid in the stomach is a determining factor in gastric inhibition. Evidence points to the fact that the free acid present in the gastric content will determine not only the initiation of inhibition but also the degree to which the contractions are changed. It is also possible that the presence of a certain quantity of the free acid in the stomach keeps the stomach in quiescence. It might be assumed on this basis that an alkaline stomach would produce hunger contractions. This does not seem to be the case, since the presence of alkali has the same inhibiting effect as acid except to a much lesser degree. In accordance with the findings of others, none of our stimuli would affect changes during the period of quiescence.

The mechanism by which this acid concentration acts to inhibit the hunger movements is probably reflex. The acid stimulates the sensory end organs in the stomach wall. The pathway of the afferent impulse in this reflex inhibition is probably partially through the sensory fibres of the vagus. In dogs with the vagi sectioned but the splanchnics intact, inhibition from psychic secretion is less marked than in the normal animal. This may be partially due to a lowered general tonus of the stomach musculature. It is sufficiently clear from experimental results that a portion of the afferent impulses involved in this reflex are through vagal sensory fibres.

Carlson [1916] in studies on five dogs with both splanchnics sectioned found an increase in tonus. Psychic or reflex inhibition of the gastric hunger contractions is greatly diminished or abolished. Stimuli which normally cause complete cessation of the hunger movements now have little or no effect. Periodicity remains but external or reflex inhibition is abolished. We may therefore assume that the efferent nervous pathway of inhibition is through the splanchnic nerves.

Having indicated the reflex pathway of normal gastric inhibition, we must also give consideration to the problem of hypnotic inhibition. If hypnosis be a central phenomenon due to increasing spread of inhibition or dissociation of the centres, is the inhibition due to (1) a direct central stimulation of the splanchnics; (2) a cutting off or dissociation of the normal impulses over the vagus diminishing the number of impulses reaching the stomach, leaving it more or less under complete splanchnic control; or (3) is the mechanism of inhibition due to a reflex set up by stimulation of the sensory nerve endings in the stomach and to the increase of the number of impulses by way of

the splanchnics? The last is the same as the normal inhibitory mechanism.

We have been unable to rule out the possibility that under hypnosis there might be a direct stimulation of thé splanchnic causing at least part of the inhibition. No experiments were attempted to test this possibility, since we were reasonably certain from other results that central stimulation did not play an important part in the inhibition so obtained. However, since there is one group who believe that hypnotism effects changes in the sympathetic nervous system, the problem ought to be investigated [McDougall, 1920].

That the vagus was not inhibited, leaving the splanchnics in full control of the gastric mechanism, was indicated by the fact that there was no drop in the general tone of the stomach during the inhibitory periods. Patterson [1933], in bullfrogs, and Carlson [1916], in dogs, have found that section of the vagus always leads to gastric hypotonia.

Luckhardt and Johnston [1924] found an increase in the acidity of the gastric juice of a hypnotised subject when a meal was suggested. Bennett and Venables [1920] claim that the rapidity of emptying time determines the hydrochloric acid content of the stomach, and indicates also that the stomach is largely under central nervous control. They state that "when the brain is filled with the conception of hunger, vigorous gastric peristalsis is the result." Bennett and Venables also claim that certain emotional states, such as anxiety, fear, and rage, cause a decrease in both motility of the stomach and acidity of the gastric content.

Kharmandarian, Platonov, and Bezchinskaia [1935] have found that under hypnosis certain pleasant psychic stimuli caused an increased tonus, while unpleasant psychic stimuli caused a weakened or scarcely perceptible peristalsis with a lowered tonus. Frick, Scantlebury, and Patterson [1935], Scantlebury and Patterson [1938], and Scantlebury [1938] have demonstrated inhibition of the contractions but no change of tonus when suggestions of eating were given to a hypnotic subject. Hellebrandt [1935] believes that the recurrent hunger cycles in the human fasting stomach are associated with fluctuations in the acidity of the gastric content. These two phases of gastric function augment and subside in unison and are dependent one on the other. No explanation is made of which is the initiating element or whether both are dependent on a third factor.

In the tracing shown in fig. 5 it may be noted that there is a latent period between the time the stimulus is given and the beginning of the inhibition. This inhibitory period is from two to four minutes. Where this occurs there usually is a partial inhibition before complete cessation of hunger movements. In the recording where only partial inhibition is produced by the stimuli, there are two to four normal contractions before this action is noted on the tracing.

Bennett and Venables [1920] and Luckhardt and Johnston [1924] have found that the total quantity, as well as an increasing total acidity of the gastric juice, is produced in a hypnotised subject. We may therefore assume that there was such an increased acidity in our subject. Carlson [1916] has found that in man, acid in contact with the gastric mucosa acts reflexly to produce inhibition of the stomach only after a latent period of from two to three minutes. Therefore, we conclude that the inhibitory effects obtained from the stomachs of our hypnotised subjects when the eating of a "hypothetical meal" was suggested was due to a reflex mechanism initiated by the increase of the acidity of the gastric juice. The acid stimulates afferent sensory nerve endings of the stomach mucosa causing a reflex, the efferent pathways of which are the branches of the splanchnic to the stomach.

Conclusions.

1. Hypnosis is valuable as an aid in scientific study since it permits the regulation of the number, kind, and intensity of stimuli used.

2. The literature fails to reveal any animal in which the so-called "Animal hypnosis" permits rapport. Therefore, animal studies are of little value in psychophysiology. This phase needs further study.

3. Normal sleep does not diminish the height of the hunger contractions.

4. The process of hypnotising a subject may diminish the strength of the hunger movements, but does not abolish them or change their rhythm.

5. The effect of the suggestion of eating a hypnotic meal depends upon where in the period of contraction the suggestion is made.

   (*a*) If the "meal" is suggested during the first two-fifths of the hunger period there is a temporary inhibition of the gastric movements. When the suggestion is stopped the contractions return to normal vigour, and a normal incomplete tetanus ending may occur.

   (*b*) If the suggestions are continued into the second two-fifths of the normal hunger period there is a complete cessation of gastric motility which continues to a normal quiescent period.

   (*c*) We were unable to inhibit the stomach movements during the period of incomplete tetanus.

6. We were not able to elicit any contractions during a period of quiescence.

The authors wish to express thanks to Mr. Harvey L. Frick for his splendid assistance, acting as operator and subject in part of these experiments.

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THE VASOCONSTRICTOR ACTIVITY ACQUIRED BY DEFIBRINATED BLOOD DURING PERFUSION OF THE ISOLATED KIDNEY OF THE DOG.

By W. J. O'Connor (Beit Memorial Research Fellow), E. B. Verney, and Marthe Vogt.[1] From the Pharmacology Laboratory, Cambridge.

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In a recent paper [Verney and Vogt, 1938] report was made of the fact that when a loop of dog's small intestine was perfused by one heart-lung preparation and a kidney by another heart-lung preparation, transposition of the kidney to the heart-lung-gut circuit was followed by a fall in the perfusion flow through the gut. Our object now is to describe experiments which, on the one hand, demonstrate the specific rôle played by the kidney in this phenomenon, and, on the other, discover certain characteristics of the response itself.

Methods.

The apparatus used in the majority of experiments was essentially that described by Canny, Verney, and Winton [1930] for the double heart-lung-kidney preparation (see fig. 1 of their paper). A few modifications proved necessary, partly to ensure constancy in the temperature and pressure of the blood feeding the organs in spite of changes in circulation and variations in vascular tone consequent upon the switching of an organ from one circulation to the other, partly to provide for the perfusion of a third organ and for its transposition from one circulation to the other at will. The connexion between the arterial supplies to the organs and that between their venous returns were therefore immersed in the thermostat, and the organs were supported in jacketed funnels and protected from draught by pieces of cellophane. Moreover, the blood feeding the organs was sent through three coils instead of one before it reached the cannulæ, thereby making one more

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modification necessary, since the resistance of the three coils was so large that the arterial pressure as measured at the brachiocephalic cannula ceased to run parallel with the perfusion pressure at the organ whenever the amount of tissue supplied by one heart-lung was changed, and with it the resistance to perfusion. Each perfusion cannula, therefore, was connected by a T-piece with one of a series of membrane manometers recording on a kymograph, and the changes in perfusion pressure which occurred when an organ was transposed from one circuit to the other were immediately compensated by appropriate

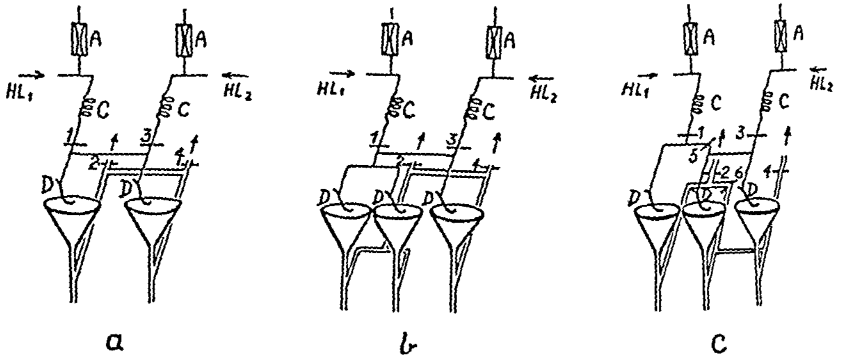

FIG. 1.—Diagrams of the arterial supplies to, and venous returns from organs perfused by heart-lung preparations. Arterial blood from the one heart-lung (HL_1) or the other heart-lung (HL_2) passes in part through the variable peripheral resistance A to the venous reservoir, in part through the warming coil C to the arterial cannula D of the perfused organ. The venous blood from the organ drains from the funnel into the venous reservoir of the heart-lung. Circuit *a*: one organ is connected to the left, and another to the right arterial cannula. Either organ can be perfused separately by the heart-lung of the same side or, in parallel with the other organ, by either heart-lung. Circuit *b*: two organs are perfused by HL_1, one by HL_2. The interconnexions of the arterial supplies and venous returns allow the left and central organs to be perfused by HL_1 or, in parallel with the third organ, by either heart-lung. Circuit *c*: two organs are perfused by HL_1, one by HL_2. In addition to the possibilities presented by circuit *b*, this circuit allows the central organ alone to be switched to and from HL_2. The numerals 1 and 2, 3 and 4, 5 and 6 represent the positions in which clamps may be placed to effect the desired transfer of the perfused organ from one circuit to the other.

adjustment of the peripheral resistances of the heart-lung preparations. Each membrane manometer, moreover, was connected in parallel with a mercury manometer of narrow bore whereby the actual pressure at the corresponding perfusion cannula was continuously revealed.

In the course of our work we have used three types of circuit and these are shown diagrammatically in fig. 1. On the left-hand side of this figure are given arterial and venous connexions which allow, on the one hand, the separate perfusion of each of two organs, and, on the other, the transposition of one organ to the contralateral heart-lung and consequent perfusion in parallel with the other organ. Fig. 1, *b* shows a simple extension of this arrangement in that two organs perfused in parallel are substituted for one in the circuit outlined in

fig. 1, *a*. In fig. 1, *c* connexions for the perfusion of three organs are again given, but here the arterial supplies and venous returns are so arranged that in addition the central organ alone can be transposed from the left- to the right-hand heart-lung preparation and *vice versa.*

The dogs used in our experiments were all anæsthetized with chloralose (0·11 g./kg. in saline) injected directly into the malleolar vein; and freshly defibrinated dog's blood filtered through four layers of fine muslin was used as the perfusate. The heart-lung preparations were made in the usual manner, and each was ventilated with warmed and moistened air by the respiration pump designed by Starling [1926]. When the preparations had been completed, the systemic outputs were diverted to the contralateral venous reservoirs for some time to ensure adequate mixing of all the blood before the two circuits were separated preparatory to the connexion of the perfused organs. The left kidney was usually chosen for perfusion, and it was carefully freed from its peritoneal connexions before its rapid excision and attachment to the perfusion cannula. In order to obtain a loop of gut for perfusion, the superior mesenteric artery of another dog was dissected for a length of 2 or 3 centimetres; and its branches, other than those supplying the selected segment of intestine, were cut between ligatures. The selected segment—a piece of jejunum 15 to 20 cm. long—was then freed from all connexion with the body, superior mesenteric vein and artery excepted, and its lumen washed with saline by means of glass tubes tied into the ends of the loop. Other tissues selected for perfusion were prepared in a similar way for transfer at appropriate times to the heart-lung circuits, the spleen being perfused through its main artery after ligation of all accessory vessels, the hind legs through the aorta after evisceration of the animal and cross-section of the vertebral column, the front leg through the axillary artery after disarticulation at the shoulder, and the forearm through the brachial artery after division of the humerus. When the artery leading to the organ selected for perfusion had been attached to its appropriate cannula, the venous effluent was at first collected in a jar: only when the organ had been well irrigated with the perfusate was the venous effluent allowed to flow back to the heart-lung reservoir. The volume of blood in each circuit lay between 0·5 and 1 litre, and to each circulation were added 5 units of insulin with 5 g. glucose and, from and including the 25th experiment, 1 mg. atropine sulphate. Experimental procedures other than those outlined above will be described in their appropriate contexts.

Results.

When a kidney perfused by one heart-lung was switched to a second heart-lung by which a loop of small intestine was being perfused (see fig. 1, *a*) vasoconstriction in the gut invariably ensued, the perfusion

flow being reduced by 20 to 70 per cent. of the original rate (average reduction in 21 experiments, 48 per cent.). Fig. 2 illustrates this phenomenon and the recovery of intestinal blood flow after the kidney had been returned to the first heart-lung. The onset of the constriction was rapid, usually noticeable within one to six minutes, and the maximal constriction was reached as a rule in 10 to 15 minutes; only occasionally (fig. 3) were longer exposures required before the lowest rate of blood-flow was attained. Further instances of this intestinal vasoconstriction may be seen in figs. 4, 5, 6, and 7, in this last figure only the constriction which occurred at C belonging to this series. In four of the experiments illustrated (figs. 2, 4, 5, and 6) the kidney was maintained in perfusion-parallel with the gut for 11 to 17 min. only, and no appreciable relaxation of the gut vessels was observed during these periods. In the experiment illustrated by fig. 7, however, perfusion of the two organs in parallel was continued for 45 min. (from 2.26 to 3.11 p.m.), and the rapid fall in rate of blood-flow through the gut was succeeded by a definite though partial recovery of flow while the gut was still being exposed to the effluent blood from the kidney. To this phenomenon we propose to return later.

In some experiments the renal artery of a freshly excised kidney was connected to a heart-lung circuit provided with arterial supply and venous return tubes as shown on the left-hand side of fig. 1, *b*. At the time when the kidney was connected, a loop of intestine was already being perfused and had been in the circuit for a period sufficiently long to allow its perfusion flow to recover appreciably from its initially low value. After the rejection of an amount of venous outflow from the kidney sufficient to ensure its adequate irrigation by the perfusate, the renal venous blood was allowed to drain into the heart-lung circuit. The effect of this procedure may be seen at A in fig. 7. The gut reacted by vasoconstriction, but a slightly longer time than in the foregoing experiments was needed for the reduction in flow to develop, probably because the blood-flow through the freshly connected kidney may only slowly attain its full value. Moreover, in two other experiments conducted in the same way, even longer periods were needed for the vasoconstriction in the gut to become established. On the other hand, the interval between exposure and reaction of the gut vessels to the effluent blood from the kidney becomes too small to be measured accurately if the gut is switched into the kidney-circuit, instead of the kidney into the gut-circuit (see fig. 10). This was done on seven occasions and the resultant reduction in flow ranged from 45 to 66 per cent., the average reduction being 55 per cent. The essential difference, therefore, between these results and those described earlier is in the speed of the response, rather than in its size. Before further analysis of the intestinal response it was necessary to perform control experiments in order to establish whether or no, under similar perfusion

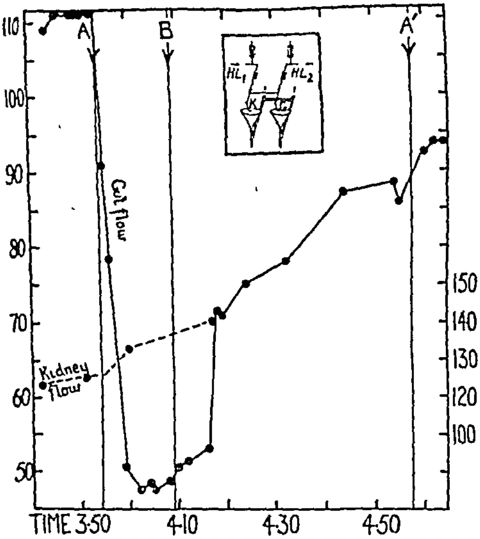

FIG. 2.—Experiment 12. Double heart-lung preparation, the perfusion circuits being as in fig. 1, *a*. A kidney is perfused by HL_1, a loop of jejunum by HL_2. At A the kidney, after having been perfused for 24 min. by HL_1, is switched to HL_2, the venous blood from the kidney beginning to drain into this circuit at 3.54 p.m. At B the kidney is switched back to HL_1. At A′ the kidney is switched a second time to HL_2. Before the period here recorded a forelimb had been switched from HL_1 to HL_2 without producing any vasoconstriction in the gut, and after 15 minutes' perfusion in parallel with the gut the forelimb was returned to HL_1. A kidney was then substituted for the forelimb in the HL_1 circuit. Perfusion pressure of both kidney and gut, 72 mm. Hg throughout. Left ordinate: perfusion flow through the gut in c.c./min.; right ordinate: perfusion flow through the kidney in c.c./min. Abscissa: time in hours and minutes. Inset: perfusion circuits.

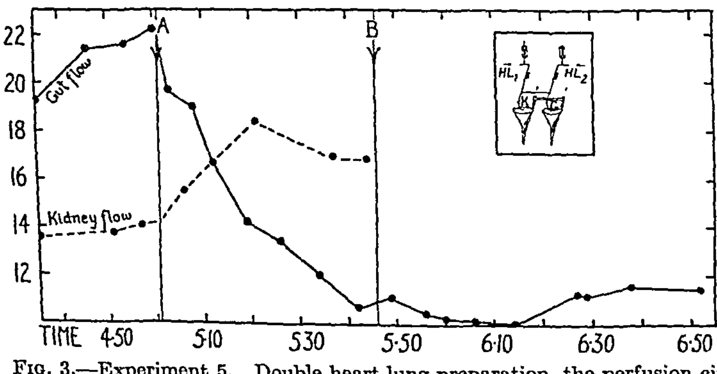

FIG. 3.—Experiment 5. Double heart-lung preparation, the perfusion circuits being as in fig. 1, *a*. A kidney is perfused by HL_1, a loop of jejunum by HL_2. At A the kidney, after having been perfused for 96 min. by HL_1, is switched to HL_2, the venous blood from the kidney beginning to drain into this circuit at 5.01 p.m. At B the kidney is switched back to HL_1. Perfusion pressure of both kidney and gut, 90 mm. Hg throughout. Ordinates and abscissa as in fig. 2, *q.v.* Inset:

flow being reduced by 20 to 70 per cent. of the original rate (average reduction in 21 experiments, 48 per cent.). Fig. 2 illustrates this phenomenon and the recovery of intestinal blood flow after the kidney had been returned to the first heart-lung. The onset of the constriction was rapid, usually noticeable within one to six minutes, and the maximal constriction was reached as a rule in 10 to 15 minutes; only occasionally (fig. 3) were longer exposures required before the lowest rate of blood-flow was attained. Further instances of this intestinal vasoconstriction may be seen in figs. 4, 5, 6, and 7, in this last figure only the constriction which occurred at C belonging to this series. In four of the experiments illustrated (figs. 2, 4, 5, and 6) the kidney was maintained in perfusion-parallel with the gut for 11 to 17 min. only, and no appreciable relaxation of the gut vessels was observed during these periods. In the experiment illustrated by fig. 7, however, perfusion of the two organs in parallel was continued for 45 min. (from 2.26 to 3.11 p.m.), and the rapid fall in rate of blood-flow through the gut was succeeded by a definite though partial recovery of flow while the gut was still being exposed to the effluent blood from the kidney. To this phenomenon we propose to return later.

In some experiments the renal artery of a freshly excised kidney was connected to a heart-lung circuit provided with arterial supply and venous return tubes as shown on the left-hand side of fig. 1, *b*. At the time when the kidney was connected, a loop of intestine was already being perfused and had been in the circuit for a period sufficiently long to allow its perfusion flow to recover appreciably from its initially low value. After the rejection of an amount of venous outflow from the kidney sufficient to ensure its adequate irrigation by the perfusate, the renal venous blood was allowed to drain into the heart-lung circuit. The effect of this procedure may be seen at A in fig. 7. The gut reacted by vasoconstriction, but a slightly longer time than in the foregoing experiments was needed for the reduction in flow to develop, probably because the blood-flow through the freshly connected kidney may only slowly attain its full value. Moreover, in two other experiments conducted in the same way, even longer periods were needed for the vasoconstriction in the gut to become established. On the other hand, the interval between exposure and reaction of the gut vessels to the effluent blood from the kidney becomes too small to be measured accurately if the gut is switched into the kidney-circuit, instead of the kidney into the gut-circuit (see fig. 10). This was done on seven occasions and the resultant reduction in flow ranged from 45 to 66 per cent., the average reduction being 55 per cent. The essential difference, therefore, between these results and those described earlier is in the speed of the response, rather than in its size. Before further analysis of the intestinal response it was necessary to perform control experiments in order to establish whether or no, under similar perfusion

was obtained in all these organs, but the responses were smaller and more sluggish than those of the jejunal vessels.

The fact having thus been established that blood leaving a perfused kidney contains a substance or substances which constrict the blood-vessels of other organs, in particular of the jejunum, it seemed to us that the properties of this substance and the conditions of its production or liberation could best be determined through some method of biological assay. Our first step, therefore, was to compare the effects of repeated exposure of the perfused gut to the renal substance, but in the aim by such means to find a method of assay we failed. The reason for this, as will appear in the results of experiments now to be described, was the failure of the gut to respond to a second exposure.

We began by transposing a kidney which had for some time been perfused by a heart-lung preparation, to a second heart-lung by which a loop of gut was being perfused (fig. 1, *a*): the usual vasoconstriction in the gut was observed. After a short exposure of the gut to the effluent blood from the kidney, the kidney was switched back to its own heart-lung and the relaxation of the gut vessels awaited: the interval needed ranged from 63 to 130 min. The kidney was then again switched to the gut circuit, but never was vasoconstriction observed to follow this manœuvre (see A′, fig. 2). In the belief that the relaxation of the gut vessels after their first exposure to the effluent blood from the kidney was due to the gradual destruction of the vasoconstrictor substance in the heart-lung-gut circuit, we attributed the absence of response of the gut vessels to a second exposure to damage of either gut or kidney during long periods of perfusion; so in order to discover, under such conditions, whether the gut vessels lost their responsiveness or the kidney ceased to produce a vasoconstrictor substance, an "old" loop of intestine was exposed to the effluent blood of a fresh kidney on the one hand, and a fresh loop of intestine to the effluent blood of an "old" kidney on the other. Two experiments will illustrate the results of these procedures. In the one experiment, that to which fig. 5 relates, the arrangement of the arterial supplies and venous returns was as shown in fig. 1, *a*. The absence of a vasoconstrictor response when the perfused kidney was switched a second time (at 2.26 p.m.) into the heart-lung-gut circuit is well seen. The kidney was then switched back to its own heart-lung circuit and replaced by a kidney freshly excised from another animal. When at D (3.19 p.m.) this second kidney was transposed to the heart-lung-gut circuit, the gut vessels again failed to constrict. At 4.04 p.m. the loop of intestine was replaced by another, and exposure of this an hour later (5.02 p.m.) to the effluent blood from the kidney failed, too, to induce a fall in its perfusion flow. In the other experiment, the results of which are given in fig. 6, the arrangement of the arterial supplies and venous returns was that shown in fig. 1, *b*, and the order of renewal of the organs was

conditions, organs other than the kidney liberated vasoconstrictor substances, and vessels other than those of the intestine were susceptible to them.

The substitution of the kidney by the spleen, by the hind legs or by a single forelimb of the dog in experiments conducted otherwise in the same way as those just described, did not produce constriction of intestinal vessels. An instance of this is given in fig. 4. In the

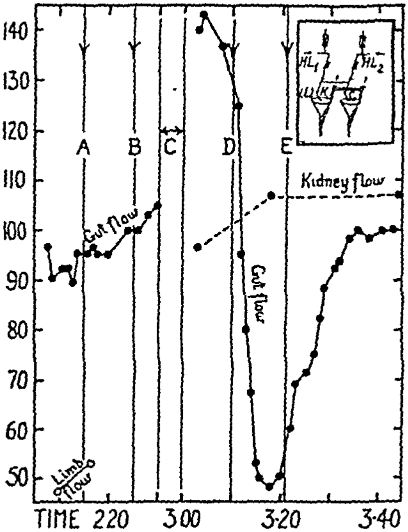

Fig. 4.—Experiment 11. Double heart-lung preparation, the perfusion circuits being as in fig. 1, *a*. A forelimb, and later a kidney, is perfused by HL_1, a loop of jejunum by HL_2. At A the forelimb is switched to HL_2, the venous blood from the limb beginning to drain into this circuit at 2.15 p.m. At B the limb is switched back to HL_1. C is a period of 30 min. during which the limb is removed and replaced by a kidney. At D the kidney is switched to HL_2, the venous blood from the kidney beginning to drain into this circuit at 3.10 p.m. At E the kidney is switched back to HL_1. Perfusion pressure of gut, forelimb and kidney *circa* 85 mm. Hg. Ordinate: perfusion flow of gut, forelimb and kidney. Abscissa: time in hours and minutes. Inset: perfusion circuits.

experiment to which this figure relates a forelimb was perfused by the one heart-lung, a loop of gut by the other, the arrangement of the arterial supplies and venous returns being as shown in fig. 1, *a*. At 2.15 p.m. the limb was switched into the heart-lung-gut circuit: no vasoconstriction in the gut ensued. When, 55 minutes later, a kidney was switched in the same way into the heart-lung-gut circuit, a rapid and strong constriction occurred in the vessels of this same loop of intestine. Lack of responsiveness by the vessels could not, therefore, account for the absence of constriction when the effluent blood from the forelimb was reaching them. Investigation of the effect of effluent blood from the kidney on organs other than the gut has been limited to forelimbs, forearms, and to pieces of large intestine: vasoconstriction

reversed. During the period of relaxation of the gut vessels from the effects of their first exposure to the effluent blood from the kidney, a freshly excised loop of gut was connected to the free cannula of the heart-lung which was already supplying the other loop. When (at 2.42 p.m.) the kidney was switched to the heart-lung by which both loops of intestine were being perfused, small diminutions in blood-flow through the loops were recorded, but these were probably entirely due to the necessity of lowering the perfusion pressure from 72 to 70 mm. Hg after the kidney had been switched into perfusion-parallel with the loops. For the remainder of the experiment the perfusion pressure was maintained at 69 mm. At 2.59 p.m. the kidney, after having been returned to its own heart-lung, was removed, and at 3.15 p.m. a kidney freshly excised from another animal was connected in its place. When 16 minutes later this kidney was switched into perfusion-parallel with the two loops of intestine no vasoconstriction was observed in either loop. We found, however (see later, p. 11), that the period of perfusion of either kidney or gut before the *initial* exposure of the gut to the effluent blood from the kidney did not affect the response to this exposure, except that slight sluggishness in its course was noticeable if the period of gut-perfusion were extended to many hours. It thus became clear that the failure of a second response by the gut vessels lay not with the "age" of either gut or kidney, but with some change in the gut vessels resulting from their first exposure to the renal substance, and that in the experiments to which figs. 5 and 6 relate, the failure of the second loop of gut to respond by vasoconstriction when the kidney was first switched into perfusion-parallel with it (G, fig. 5; D, fig. 6) was associated with the introduction of this loop into a circuit still contaminated with renal vasoconstrictor substance.

The results recorded in figs. 5 and 6 are, then, inconsistent with the view that the relaxation of the gut vessels after their first exposure to the effluent blood from the kidney is due simply to the gradual destruction of the renal vasoconstrictor substance in the heart-lung-gut circuit, but find concordant interpretation in the assumption that a single exposure of a loop of intestine to blood which has perfused the kidney is sufficient to produce in the gut a persistent vascular refractoriness. The gut vessels would thus become unresponsive to perfusion in parallel not only with the originally active but also with any freshly excised kidney. If this assumption be correct, the intestinal vessels should, after their initial constriction, relax while the kidney is retained in perfusion-parallel with the gut and is still liberating the vasoconstrictor substance. That this is indeed the case is shown by the results recorded in fig. 7. In this experiment the arrangement of the arterial supplies and venous returns was that shown in fig. 1, *b*. Perfusion of the kidney and gut in parallel began at 12.46 p.m., and was not interrupted till an hour and forty minutes later. Twenty-eight minutes, however,

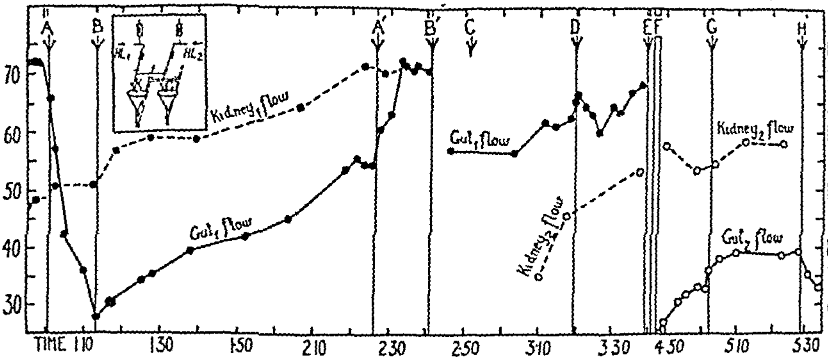

FIG. 5.—Experiment 25. Double heart-lung preparation, the perfusion circuits being as in fig. 1, *a*. A kidney is perfused by HL_1, a loop of jejunum by HL_2. At A the kidney, after having been perfused for 20 min. by HL_1, is switched to HL_2, the venous blood from the kidney beginning to drain into this circuit at 1.00 p.m. At B the kidney is switched back to HL_1. At A′ the kidney is again switched to HL_2, at B′ returned again to HL_1, and then removed. At C a fresh kidney is connected to HL_1, and at D this kidney is switched to HL_2, the venous blood from the kidney beginning to drain into this circuit at 3.19 p.m. At E the kidney is switched back to HL_1. F is an interval of 66½ min. during which the loop of gut is removed and a fresh loop prepared in a newly anæsthetised animal. This loop is connected to HL_2 at 4.04 p.m. At G the kidney is switched to HL_2, the venous blood from the kidney beginning to drain into this circuit at 5.02 p.m. At H the kidney is switched back to HL_1. The perfusion pressure of gut and kidney till 2.40 p.m. was 74 mm. Hg; from 2.40 p.m. it was 72 mm. Hg. Ordinates and abscissa as in fig. 2, *q.v.* Inset: perfusion circuits.

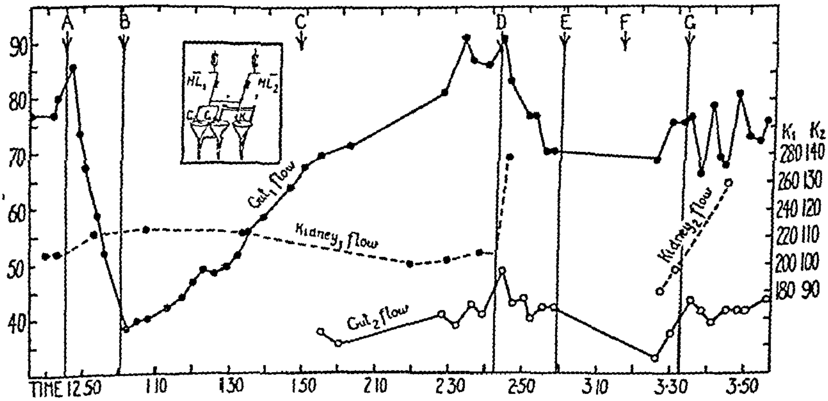

FIG. 6.—Experiment 20. Double heart-lung preparation, the perfusion circuits being as in fig. 1, *b*. A kidney is perfused by HL_2, a loop of jejunum by HL_1. At A the kidney, after having been perfused for 20 min. by HL_2, is switched to HL_1, the venous blood from the kidney beginning to drain into this circuit at 12.45 p.m. At B the kidney is switched back to HL_2. At C a second (additional) loop of jejunum is connected to HL_1. At D the kidney is switched to HL_1, the venous blood from the kidney beginning to drain into this circuit at 2.42 p.m. At E the kidney, after having been switched back to HL_2, is removed. At F a second kidney is connected to HL_2, and at G this kidney is switched to HL_1, the venous blood from the kidney beginning to drain into this circuit at 3.32 p.m. At 2.44 p.m. the perfusion pressure of the two loops of intestine was lowered from 72 to 70 mm. Hg. Left ordinate: perfusion flow of G_1 and G_2 in c.c./min.; right ordinates: perfusion flow of K_1 and of K_2 in c.c./min. Abscissa: time in hours and minutes. Inset: perfusion circuits.

is shown by the fact that when at 4.00 p.m. the kidney was again transposed to this circuit, no constriction of the intestinal vessels ensued. In another and similarly conducted experiment, vasoconstriction in a loop of gut was observed when a kidney hitherto perfused for as long as two hours and fifty minutes was transferred to the heart-lung-gut circuit. In the light of these findings, therefore, the irresponsiveness seen in figs. 5 and 6 of fresh loops of intestine to exposure to the renal effluent blood, finds reasonable interpretation in these loops having been connected with heart-lung circuits the blood of which was already contaminated by undestroyed constrictor substance from the kidney.

The experiment to which fig. 7 relates proves that prolonged perfusion of the kidney causes no appreciable decline in the constrictor effect of its venous outflow. Similarly, we have found that the "age" of the gut, *i.e.* the period of its perfusion, is equally unimportant in determining the response of its vessels. For example, in one experiment a large vasoconstrictor response was obtained in a loop of gut at its first connexion with the heart-lung-kidney circuit 4 hr. 40 min. from the beginning of its perfusion.

The conclusion to which our experiments have now led, is that the absence of a constrictor response by the intestinal vessels to their second exposure to the effluent blood from the kidney is determined not by a decline with age either in the production of constrictor substance by the kidney or in the responsiveness of gut vessels to it, but by a refractoriness contingent upon an earlier reaction between this substance and the contractile elements of the perfused vessels. The validity of this conclusion, derived from the association of results of several experiments, can be established or refuted only by an arrangement whereby three separate circuits are used, the first to perfuse one loop of gut, the second to perfuse another, and the third to perfuse a kidney. Suitable interconnecting tubes between the arterial supplies and venous returns of the three perfused organs will then permit the kidney to be switched in the first place into the circuit feeding one loop of gut, and later into that feeding the two loops of gut, the first loop having in the meantime been switched into the circuit feeding the second loop of gut. For this purpose an apparatus was assembled in which the heart-lung preparations of the previous experiments were replaced by three pump-lung circuits. A plan of the requisite apparatus immersed in a thermostat is given in fig. 8.

A systemic (1, 3, 5) and a pulmonary (2, 4, 6) pump are used for each circuit, and the corresponding venous reservoirs are shown in the figure at *a*, *c*, *e* and *b*, *d*, *f*. The six pumps [Dale and Schuster, 1928] are screwed in two rows to the table beneath the thermostat and are driven by a single motor. Each is connected by brass tubing through the bottom of the tank with its corresponding glass compression chamber. The temperature of the water in the bath is maintained uniform and constant by two stirring vanes and two

after the onset of exposure of the gut to the venous effluent from the kidney, relaxation of the gut vessels set in, and during the succeeding hour the gut-flow increased to reach a figure larger even than the initial value. Meanwhile (at 1.55 p.m.) a fresh loop of intestine had been connected to heart-lung 2, and stress must be laid on the fact that, in contrast with the experiments of figs. 5 and 6, the circuit to which the new loop of gut was connected had remained throughout completely

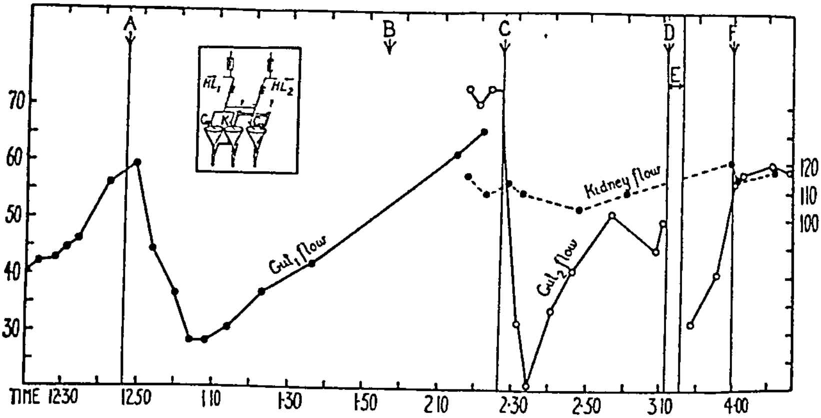

FIG. 7.—Experiment 28. Double heart-lung preparation, the perfusion circuits being as in fig. 1, *b*. A loop of jejunum is perfused by HL_1, and at A a kidney is attached to the free cannula of this circuit. At B a second loop of jejunum is connected to HL_2. At C the kidney is switched to HL_2, the arterial supply and venous return tubes of the first loop of gut having been previously clamped; and the venous blood from the kidney begins to drain into the HL_2 circuit at 2.26 p.m. At D the kidney is switched back to HL_1. E is a period of 34 minutes, during which most of the blood in HL_2 is replaced by freshly defibrinated blood. This, as is usual, is followed by an evanescent intestinal vasoconstriction, the recovery from which is shown. At F the kidney is again switched to HL_2, the venous blood from the kidney beginning to drain into this circuit at 3.59 p.m. Perfusion pressure, 80 mm. Hg throughout. Left ordinate: perfusion flow through the first and second loops of gut in c.c./min.; right ordinate: perfusion flow through the kidney in c.c./min. Abscissa: time in hours and minutes. Inset: perfusion circuits.

uncontaminated by effluent blood from the kidney. At 2.26 the kidney was switched over to this heart-lung-gut circuit: the ensuing strong and rapid vasoconstriction showed that the kidney was able then to liberate the vasoconstrictor principle in as effective an amount as an hour and forty minutes earlier. Exposure of this second loop of intestine to the kidney extended over a period of 45 minutes: the gut vessels, after their initial constriction, began to relax within 14 minutes, and the blood-flow had recovered to more than half its original value within 30 minutes of the initial exposure of the gut to the renal effluent blood. The kidney was then returned to heart-lung 1 circuit, and most of the blood in heart-lung 2 circuit was replaced by defibrinated blood from a newly bled animal. The failure of this manœuvre to abolish the refractoriness of the gut vessels to the renal vasoconstrictor principle

is shown by the fact that when at 4.00 p.m. the kidney was again transposed to this circuit, no constriction of the intestinal vessels ensued. In another and similarly conducted experiment, vasoconstriction in a loop of gut was observed when a kidney hitherto perfused for as long as two hours and fifty minutes was transferred to the heart-lung-gut circuit. In the light of these findings, therefore, the irresponsiveness seen in figs. 5 and 6 of fresh loops of intestine to exposure to the renal effluent blood, finds reasonable interpretation in these loops having been connected with heart-lung circuits the blood of which was already contaminated by undestroyed constrictor substance from the kidney.

The experiment to which fig. 7 relates proves that prolonged perfusion of the kidney causes no appreciable decline in the constrictor effect of its venous outflow. Similarly, we have found that the "age" of the gut, *i.e.* the period of its perfusion, is equally unimportant in determining the response of its vessels. For example, in one experiment a large vasoconstrictor response was obtained in a loop of gut at its first connexion with the heart-lung-kidney circuit 4 hr. 40 min. from the beginning of its perfusion.

The conclusion to which our experiments have now led, is that the absence of a constrictor response by the intestinal vessels to their second exposure to the effluent blood from the kidney is determined not by a decline with age either in the production of constrictor substance by the kidney or in the responsiveness of gut vessels to it, but by a refractoriness contingent upon an earlier reaction between this substance and the contractile elements of the perfused vessels. The validity of this conclusion, derived from the association of results of several experiments, can be established or refuted only by an arrangement whereby three separate circuits are used, the first to perfuse one loop of gut, the second to perfuse another, and the third to perfuse a kidney. Suitable interconnecting tubes between the arterial supplies and venous returns of the three perfused organs will then permit the kidney to be switched in the first place into the circuit feeding one loop of gut, and later into that feeding the two loops of gut, the first loop having in the meantime been switched into the circuit feeding the second loop of gut. For this purpose an apparatus was assembled in which the heart-lung preparations of the previous experiments were replaced by three pump-lung circuits. A plan of the requisite apparatus immersed in a thermostat is given in fig. 8.

A systemic (1, 3, 5) and a pulmonary (2, 4, 6) pump are used for each circuit, and the corresponding venous reservoirs are shown in the figure at *a*, *c*, *e* and *b*, *d*, *f*. The six pumps [Dale and Schuster, 1928] are screwed in two rows to the table beneath the thermostat and are driven by a single motor. Each is connected by brass tubing through the bottom of the tank with its corresponding glass compression chamber. The temperature of the water in the bath is maintained uniform and constant by two stirring vanes and two

hot points, the latter being controlled through series resistances. The oxygenated blood of each circuit divides between a variable resistance (A) and a

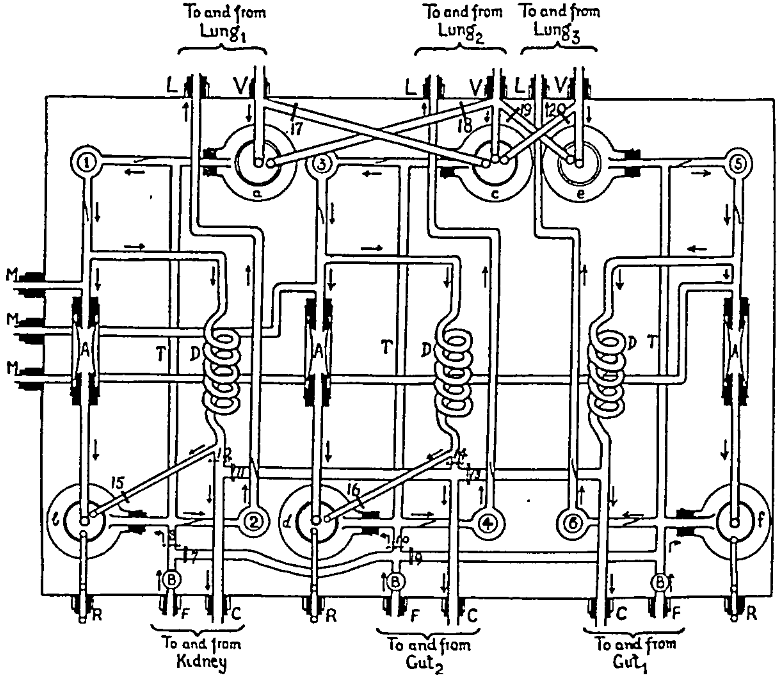

FIG. 8.—Triple pump-lung perfusion system. The figure is a diagrammatic plan of the thermostat and its contents. 1–6 represent the compression chambers of six Dale-Schuster pumps, the valves of which are indicated in the tubes leading to and from the compression chambers. *a*, *c*, and *e* are the systemic reservoirs; *b*, *d*, and *f* the pulmonary. Blood from the lungs drains into the systemic reservoir through the tubes V, and blood from the perfused organs drains into the pulmonary reservoirs through the tubes F. The tubes L are connected with the pulmonary arterial cannulæ, the tubes C with the arterial cannulæ of the perfused organs. The systemic variable resistances are shown at A, the warming coils at D, and the interconnexions between the pulmonary and systemic reservoirs of each circuit are shown at T. The oblique tubes 17, 18, 19, and 20 allow the blood in the three circuits to be adequately mixed: these tubes are closed before the kidney is connected. Each of the two small tubes 15 and 16 is opened when the organ perfused by its respective circuit is transposed temporarily to another: stagnation of blood in the coils D is thereby prevented. Bubble traps on the course of the venous return tubes from the funnels supporting the perfused organs are shown at B, and indicators of the level of blood in the pulmonary reservoirs are shown at R. The tubes M lead to three mercury manometers. An air-cushion (not indicated in the figure) is inserted between the systemic pump and variable resistance (A) of each circuit. When the clamps 7, 11, 9, and 13 are closed the three circuits are entirely separate. The transfer of clamp 7 to position 8 and of clamp 11 to position 12 transposes the organ perfused by the left-hand circuit to perfusion-parallel with that perfused by the central circuit; the transfer of clamp 9 to position 10 and of clamp 13 to position 14 transposes the organ perfused by the central circuit to perfusion-parallel with that perfused by the right-hand circuit. When both transfers are made all three organs are perfused in parallel by the right-hand circuit. Muslin filters are suspended in the necks of the systemic reservoirs *a*, *c*, and *e*.

warming coil (D) through which it is conducted to the arterial cannula of the organ supported in a jacketed funnel outside the thermostat. A mercury

manometer is connected by a tube (M) with the high pressure side of each variable resistance (A), and the actual pressure at each of the perfusion cannulæ is disclosed by attaching its lateral limb to a recording membrane manometer. The blood leaving the organ and that passing through the variable resistance drain into the pulmonary reservoir (*b*, *d*, *f*) whence the blood is pumped into the pulmonary artery of one of three separate lungs. The lung is prepared for perfusion by bleeding a chloralosed dog, tying cannulæ into the pulmonary artery and left auricle, inserting a tracheal cannula, ligating one lung at its root and irrigating the other with defibrinated blood. The thorax is then isolated and transferred to a chamber which is later sealed by a plate glass lid for the purpose of negative pressure ventilation.

The method of negative pressure ventilation is essentially that described by Daly and Thorpe [1930] (see also Berry and Daly [1931]). The chamber is connected with a "vacuum cleaner," and rhythmic changes in negative pressure are produced by a valve consisting of a piston which slides up and down an open brass cylinder fitted with a side hole leading directly into the chamber, which is thus opened intermittently to the exterior. The three pistons are connected to a crank shaft which revolves at a frequency of 17 per min., and the variations in pressure within each chamber are revealed by a water manometer, and controlled by the adjustment of a screw clip on a wide rubber tube leading from the outside to the inside of the chamber. The air within is moistened by the introduction of steam, and warmed by a carbon lamp which is controlled by a variable resistance in series with it.

The pulmonary arterial and left auricular cannulæ are connected through the wall of the chamber with the pulmonary supply (L) and return (V) tubes of the perfusion circuit, this having meanwhile been filled with defibrinated blood. The chamber is then sealed with the glass lid and the lung rhythmically ventilated. The blood leaving the lung passes a muslin filter in the neck of the systemic reservoir, whereby the risk of small clots gaining access to the systemic circuit from incompleteness in initial irrigation of the lung is avoided. The output of the pulmonary pump is adjusted to be in excess of that of the systemic, the wide connexion (T, fig. 8) between the pulmonary and systemic reservoirs of each circuit preventing the banking of blood in the latter.

The connexions between the arterial supplies and venous returns of the perfused organs are as shown in the figure, and a small shunt (15, 16, fig. 8) branches from the distal end of the left and of the central warming coil to the corresponding pulmonary reservoir: this is opened while the organ normally fed by that circuit is temporarily connected with another, and stagnation of blood in the warming coil is thereby prevented. Before the organs are connected, the blood of the three pump-lung circuits is thoroughly mixed by diverting the blood from the lung in the left circuit (fig. 8) to the systemic reservoir of the central circuit and *vice versa*, and following this by a similar procedure with the central and right circuits. The blood contents of the three circuits are then adjusted so that that of the right is greater than, and that of the left less than that of the central circuit, since during the experiment blood will be drained once from the central to the left circuit, once from the right to the central, and once from the right to the left circuit. The circuits are now separated by allowing the blood from each lung to drain into its corresponding systemic reservoir and by

closing the interconnexions of the arterial supply tubes to the perfusion cannulæ and those of the venous return tubes from the jacketed funnels.

In the experiment illustrated by fig. 9, this stage was reached at 3.15 p.m. At 3.22 a loop of gut (G_1) was connected to the right circuit,

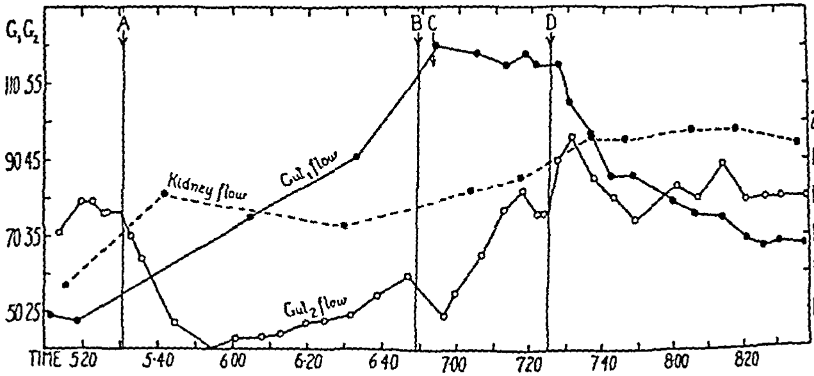

FIG. 9.—Triple pump-lung preparation. One loop of jejunum (G_1) is perfused by the right-hand circuit, a second loop (G_2) by the central and a kidney by the left-hand circuit (see fig. 8). At A the kidney is switched to the central circuit, the venous blood from the kidney beginning to drain into this circuit at 5.31 p.m. At B the kidney is switched back to the left-hand circuit. At C the loop of gut G_2 is switched from the central to the right-hand circuit, the venous blood from the loop beginning to drain into this circuit at 6.53 p.m. At D the kidney is switched to the right-hand circuit, the venous blood from the kidney beginning to drain into this circuit at 7.25 p.m.: all three organs are now being perfused in parallel by the right-hand circuit. Perfusion pressure 91 mm. Hg throughout. 1 mg. atropine sulphate was added to each circuit before the organs were connected. Left ordinates: perfusion flow of G_1 and G_2 in c.c./min.; right ordinate: perfusion flow of kidney in c.c./min. Abscissa: time in hours and minutes.

at 4.17 a second loop (G_2) was connected to the central, and at 4.25 p.m. a kidney was connected to the left circuit. When the blood-flow through the "younger" gut (G_2) was fairly steady, the kidney was transposed to the central circuit by moving the clamp shown at 11 (fig. 8) to the position 12, and, after a period which allowed washing of the renal vascular bed with more than 100 c.c. of blood, the clamp shown at 7 was transferred to position 8 on the venous return to the left circuit. The renal effluent blood was now flowing into the central circuit (A, fig. 9). Vasoconstriction in the gut ensued, and, as will be seen in the figure, this was evident five minutes after the kidney was transposed to the circuit, and was maximal twenty minutes later. The gut vessels then gradually relaxed while the kidney was retained in the circuit. The course of the reaction of these vessels to the effluent blood from the kidney is thus the same with pump-lung as with heart-lung preparations. When the vessels of the gut (G_2) had relaxed somewhat, the kidney was returned to its original circuit, and four minutes later (at 6.53) the gut was transposed to the right-hand circuit which was already feeding the "older" loop (G_1). This was accom-

plished by moving the clamp shown at 13 (fig. 8) to the position 14, and, after a thorough washing of the vascular bed of the gut by uncontaminated blood in the right-hand circuit, the venous effluent was diverted to this circuit by transferring the clamp shown at 9 (fig. 8) to the position 10. Perfusion of the two loops of gut by this circuit was continued until the "younger" gut (G_2) was exhibiting approximately the same perfusion flow as at the beginning of the experiment; the kidney was then transposed to the same circuit by again moving the clamp at 11 (fig. 8) to position 12 and, a little later, the clamp at 7 to position 8. The "older" (G_1) and the "younger" gut (G_2) were thus exposed almost simultaneously to the effluent blood from the kidney: there was no response by the vessels of the latter (G_2) which had been exposed once before to the renal vasoconstrictor substance, whereas those of the former (G_1) constricted to such a degree that the percentage fall in perfusion flow was approximately the same as that given by the "younger" gut (G_2) at its first exposure nearly two hours earlier. The circulating blood volumes were approximately equal when the two exposures were made. The conclusions, therefore, are obligatory, first that the kidney liberates a vasoconstrictor substance continuously during long periods of perfusion; second, that prolonged perfusion does not in itself appreciably affect the responsiveness of the intestinal vessels to this substance; and third, that exposure of these vessels to the vasoconstrictor substance produces in them a persistent refractoriness to it. This refractoriness is expressed both in the failure of the gut-vessels to respond to a second exposure and in their relaxation during prolonged exposure.

Now the establishment of these facts allows certain deductions to be drawn with regard to the stability of the vasoconstrictor substance in defibrinated blood. At E in fig. 5 (3.40 p.m.) the kidney was switched out of the gut circuit, and 24 minutes later (at 4.04 p.m.) a fresh loop of gut was substituted for the old. The fact that this fresh loop was found to be refractory when tested an hour later shows that the renal substance must still have been present in the blood of the gut circuit at least 24 minutes after its source of supply had been withdrawn. Again, at B in fig. 6 (1.01 p.m.) the kidney was switched out of the gut circuit, and 45 minutes later (at 1.46 p.m.) a fresh loop of gut (G_2) was connected to the same circuit. This loop, as we have already seen, was refractory when, at 2.42 p.m., the test was made. The blood of the gut circuit therefore must, in this instance, have contained the renal substance at least 45 minutes after its source of supply had been withdrawn, and at a time when the blood-flow through the first loop of gut (C, fig. 6) had recovered to a value which was only 16 per cent. less than the original rate.

The experiment illustrated in fig. 9 shows, in addition to the points already made, that the refractory state, if fully developed, cannot be

eluted by irrigation of the gut-vessels with blood completely uncontaminated with effluent blood from the kidney. A few experiments have therefore been conducted with a view, first, to ascertaining whether the same holds for short exposure to the renal effluent, and, second, to tracing the development of the refractory state during the exposure. In these experiments, contrary to the procedure usually adopted, the gut was switched to and from the kidney circuit, thereby being exposed suddenly to a high concentration of renal substance but for a fully controlled and strictly delimited period. In two such experiments (heart-lungs were used as the perfusion systems) the arrangement of the arterial supplies and venous returns was that given in fig. 1, *a*. When the loop of jejunum was switched into the kidney circuit, precautions were taken, by transferring the venous return clamp before the arterial supply clamp, to prevent any blood in the kidney circuit from draining into the gut circuit. The response of the gut was sharp and rapid. In the one experiment, after 14 minutes' perfusion in parallel and at a time when the maximal degree of constriction had already been passed, the gut was switched back to its own heart-lung, and on this occasion the arterial supply clamp was transferred before the venous return clamp, an interval of some minutes being allowed to elapse between these manœuvres to ensure that none of the blood in the heart-lung-kidney circuit reached the heart-lung-gut circuit. After 73 minutes' perfusion in its own circuit with blood uncontaminated by renal effluent, the loop was again switched to the heart-lung-kidney circuit: no vasoconstrictor response was obtained. In the other experiment a similar procedure was adopted: the first response to renal substance was a reduction in perfusion flow by 40 per cent. of the original rate; the exposure continued for 23 minutes, during the last eight of which the gut flow was not appreciably changed; after 26 minutes' perfusion of the gut in the uncontaminated heart-lung circuit a second exposure gave a reduction of 10 per cent. only, and no effect was seen at a third trial after an interval of another 43 minutes. These results suggested to us that the refractoriness of the gut-vessels became absolute, and incapable of dislodgment by prolonged washing with blood completely free from renal effluent, at some time during the plateau of maximal vasoconstriction. If, however, the exposure is terminated before the vessels are maximally constricted, a partial refractoriness is detectable by exposing them a second time to the substance liberated by the kidney, as the following experiment shows. The circuit was that outlined in fig. 1, *c*, and the results of the experiment are given in fig. 10. Two loops of intestine were dissected; the first (G_1) was connected to heart-lung 1 at 1.32 p.m., the second (G_2) to the *same* heart-lung at 1.50. At 2.13 a kidney was connected to heart-lung 2. At 2.48 the "younger" gut only (G_2) was transposed to the heart-lung-kidney circuit for a period of 11 minutes, and then returned

to heart-lung 1 by an appropriate transfer of its arterial clamp, the transfer of the venous clamp being delayed for a further four minutes to ensure that heart-lung 1 remained uncontaminated by blood from the kidney circuit. As is seen in fig. 10, a constriction of the gut-vessels

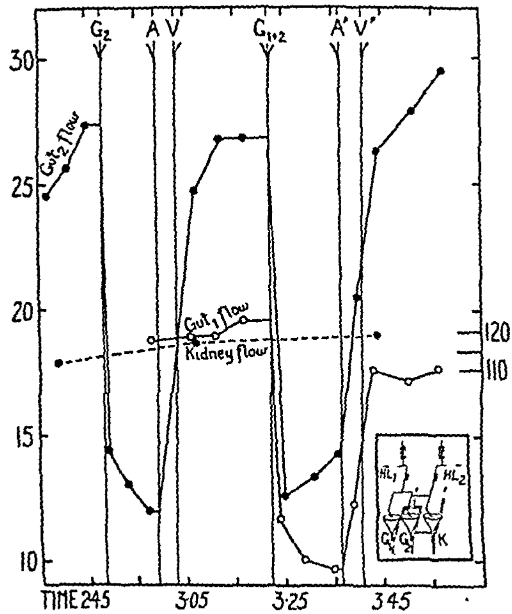

Fig. 10.—Experiment 33. Double heart-lung preparation, the perfusion circuits being as in fig. 1, c. One loop of jejunum (G_1) is perfused through the left-hand cannula of HL_1, a second loop (G_2) through the remaining cannula of HL_1. A kidney is perfused by HL_2. At G_2 the second loop of jejunum is switched to HL_2. At A the arterial cannula of G_2 is switched back to HL_1, and at V the venous return of G_2 is switched back to HL_1. At G_{1+2} both loops of jejunum are switched to HL_2. At A′ the arterial cannulæ of both loops are switched back to HL_1, and at V′ the venous returns of both loops are switched back to HL_1. Perfusion pressure 65 mm. Hg throughout. Left ordinate: perfusion flow through each loop of gut in c.c./min.; right ordinate: perfusion flow through kidney in c.c./min. Abscissa: time in hours and minutes. Inset: perfusion circuits.

occurred, large and almost instantaneous at the beginning of, and progressive during, the period of their exposure, to be followed by rapid and complete relaxation when the gut was returned to its original circuit. At 3.22 p.m., G_1 and G_2 were together switched into the kidney circuit: the "older" (G_1) responded just as rapidly and with a similar percentage reduction in flow as had the "younger" (G_2) before, the constriction of its vessels being also progressive during the period of exposure. Not so G_2: although its vessels constricted almost as well as at their first exposure, relaxation began while the gut was still in the heart-lung-kidney circuit, an indication that a state of partial refractoriness had developed during the first exposure and was augmenting at the time of the second.

We thought it desirable to test the possibility of the lungs playing an essential rôle in the formation of the vasoconstrictor substance, and to this end a system involving the left and central circuits of fig. 8 was used, two oxygenators of the Hooker [1915] type being substituted for the lung and lung-boxes there employed for oxygenating the blood. In each oxygenator the surface on to which the blood was spread was that of a cylinder 51 cm. high and 25 cm. in diameter, and the oxygen, passing through warm water on its way to the cylinders, was kept at a temperature of 37° as it entered them. When the circuits had been filled with defibrinated blood, this was driven into the oxygenators by the "pulmonary" pumps 2 and 4 (fig. 8) whence it flowed by gravity into the systemic reservoirs *a* and *c*. As in the experiment illustrated by fig. 9, the outputs of the "pulmonary" pumps were adjusted to be in excess of those of the systemic. A loop of intestine and a kidney were now dissected and perfused each by a separate circuit. Owing to the high vasotonin content of the blood in the absence of the lungs [Eichholtz and Verney, 1924], the blood-flows through kidney and gut were small (12·0 c.c. and 20·5 c.c./min. respectively at a perfusion pressure of 119 mm. Hg); but when the kidney was transferred to the gut circuit, the flow through the gut diminished to reach a minimum of 8 c.c./min. 18 min. after the transfer, and then increased to reach its original value within the next hour. Because of the smallness of these flows the experiment was repeated but with preliminary passage of the blood through lungs in order to remove vasotonins. As soon as the circuits had been filled, the heart and lungs of one of the bled dogs were excised, a cannula was tied into the pulmonary artery, a clamp applied to the auriculo-ventricular groove, the left auricle freely opened, and the lungs were irrigated with defibrinated blood. The pulmonary artery was then connected with the arterial cannula of the left pump-oxygenator circuit and the blood from the left auricle drained through a muslin filter into the "pulmonary" reservoir of the same circuit. The blood of both circuits was passed continuously through the lungs by allowing that from the oxygenator of the one circuit to flow into the systemic reservoir of the other. The lungs meantime were being ventilated by a Starling pump, and when they had been perfused at a pressure of 35 mm. Hg for from 50 to 80 min., a loop of intestine was connected to the middle circuit. The lungs were then removed, the circuits separated, and a kidney was connected to the left-hand circuit. The blood-flows through both kidney and intestine were large, and vasoconstriction in the latter occurred as the result of transposing the kidney to the gut circuit; in one instance, for example, the gut-flow fell in 16 min. from 63·8 to 41·8 c.c./min. as a result of this procedure. Relaxation of the vessels then began, and a second exposure to renal effluent failed to produce constriction again. Since these results accord with those of the heart-lung and pump-lung perfusion systems,

the lungs can play no concurrent rôle in the phenomenon under study.

We now decided to determine whether or no the phenomenon appeared when blood made incoagulable by heparin was substituted for defibrinated blood as perfusate. Experiments were therefore performed with the technique as just described, the only modification being the use throughout of blood rendered incoagulable by the addition of heparin [1] in a concentration of 10 mg./100 c.c. blood. When the kidney was transposed to the gut circuit, no constriction of the intestinal vessels ensued; on the contrary, a dilatation was recorded. The failure of vasoconstriction to appear under these conditions was not due to simple intervention by heparin in suppressing the response, since the typical constriction and ensuing refractoriness of the gut-vessels were found when the perfusate was defibrinated blood to which heparin in a concentration of 10 mg./100 c.c. blood had been added. Under perfusion conditions, therefore, the production by the kidney of the vasoconstrictor agent in effective amount, or the responsiveness of the gut-vessels to it, appears to be determined by the use of defibrinated blood as perfusate.

The technique at our disposal offered the possibility of discovering whether the constrictor substance in the venous effluent from a kidney perfused with defibrinated blood acted on the intestinal vessels directly or on the terminations of their sympathetic nerves. To this end the following operation was performed on a bitch under ether anæsthesia and with full surgical precautions:—

The superior mesenteric artery and vein were exposed through a mid-abdominal incision and thoroughly freed and cleaned for a distance of about two centimetres. All the nerve fibres, lymph vessels and connective tissue in the region were then divided between ligatures, the mesenteric artery and vein alone being spared. The animal made a good recovery although profuse diarrhœa developed the day after the operation, and this persisted till the day of the perfusion experiment one month later. The animal remained, however, in lively health throughout, but became more and more emaciated in spite of good appetite. Subcutaneous injection of atropine sulphate in a dose of 2 mg./day seemed to check the diarrhœa somewhat in the early days, but later was without apparent effect. Water and food intake caused prompt emptying of the bowels, as had been observed before by Verney and Vogt [1938] in a dog in which all the prevertebral ganglia had been excised.

A month after the operation a loop of this animal's jejunum was perfused, and the effect of the renal venous effluent on its blood-flow determined in the manner described on p. 18. Two pump-oxygenator circuits were used, and the defibrinated blood was circulated through a pair of lungs for over two hours before separation of the circuits and

[1] The heparin was that issued by The British Drug Houses Ltd., and prepared by them in co-operation with Dr Jorpes. We found that with concentrations less than 10 mg./100 c.c. blood, small amounts of fibrin might be deposited on the walls of the apparatus in long lasting perfusion experiments.

connexion of the kidney. Fig. 11 is a graph of the results obtained. The loop of gut was being perfused in the one pump-oxygenator circuit, the kidney in the other. At the arrow the kidney was transposed to the gut circuit: the intestinal vessels, which had been slowly dilating, now began to constrict, and after 15 minutes' perfusion of the two *organs in parallel the blood-flow through the gut* was reduced by as much as 44 per cent. Relaxation of the vessels then began and pro-

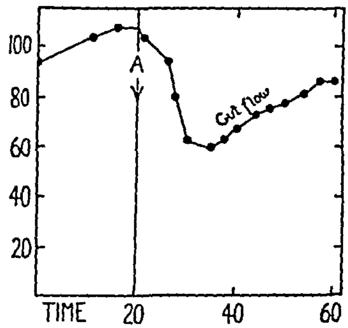

Fig. 11.—Double pump-oxygenator preparation. A loop of jejunum (denervated one month previously) is perfused by one pump-oxygenator, a kidney by the other. At A the kidney is switched to the circuit perfusing the gut. Ordinate: perfusion flow through the gut in c.c./min. Abscissa: time in minutes.

gressed in spite of the continued presence of the kidney. The degree and course of the response differ in no apparent way from those found when a loop of intestine from a normal dog is used: the vasoconstrictor substance produced by the perfused kidney, therefore, acts either on the myoneural junctions or on the plain muscle of the vascular bed.

Particular note may be made of the fact that in the later experiments atropine sulphate (1 mg.) was added to the blood of each circuit (0·5 to 1 litre). Thus the vasoconstriction of the gut-vessels in the experiments of figs. 5, 7, 9, and 10 occurred in the presence of atropine, while in those of figs. 2, 3, 4, and 6 it occurred in the absence of this drug. Obviously the response is not essentially changed by atropine in a concentration of 0·1–0·2 mg./100 c.c blood.

Discussion.

The experiments described in the foregoing pages were originally planned with a view to the possibility of their results having a bearing on the phenomenon of hypertension from renal ischæmia. The experiments have demonstrated the liberation of a vasoconstrictor substance by the dog's kidney when isolated and perfused with oxygenated defibrinated blood, the liberation occurring irrespective of whether the kidney is perfused by a heart-lung, pump-lung, or pump-oxygenator system. The substance appears to be liberated by the kidney over

long periods of perfusion (*e.g.* 3 to 5 hours), and the blood-vessels of the perfused loop of jejunum which we have used as an indicator of this substance are still responsive after perfusion periods of similar duration. The possibility, however, of using the response of the gut-vessels as a measure of the amount of vasoconstrictor substance liberated by a kidney as the result of variations in the perfusion pressure and in the composition of the defibrinated blood supplied to it, was denied by the finding that a single exposure of the gut-vessels to the effluent blood from the kidney created in them a persistent refractoriness to subsequent exposures. Our experiments indicate that the more quickly the gut-vessels constrict when the kidney is switched into the gut circuit, the more rapidly do they relax and exhibit refractoriness to further exposure when the kidney has been switched out of the gut circuit (*cf.* figs. 2 and 4 with figs. 5 and 6): when the speed of constriction has been very slow, no appreciable recovery at all may be seen (fig. 3). Moreover, the onset and course of relaxation of the gut-vessels while exposure to the effluent blood of the kidney is maintained—another expression of refractoriness—is also a function of the speed of initial constriction: when vasoconstriction has occurred rapidly, as is shown, for example, in fig. 7 at C, recovery of flow is quickly noticeable; whereas when it has occurred slowly (fig. 7 at A, fig. 9 at A) the course of recovery is prolonged. If we assume, in the absence of a means of assaying the concentration of the vasoconstrictor substance in blood, that the speed of constriction of the gut-vessels on their first exposure to the effluent blood from the kidney is directly proportional to the amount of constrictor substance in the blood which reaches them, our results indicate that sufficiently low concentrations of this substance may well produce a definite increase in vascular tone the persistence of which is unmolested by the refractoriness which follows exposure of the vessels to higher concentrations. This refractoriness, therefore, cannot be justified as a phenomenon which in itself excludes the possibility of identity or close chemical relationship between the substance liberated by a perfused kidney and that liberated [Goldblatt, 1937] by an ischæmic kidney in the otherwise normal animal. On the other hand, it would be misleading to assume any connexion in cause between the phenomenon of persistent hypertension in the normal animal following obstruction of the arterial supply to the kidney and the phenomenon of constriction of the gut-vessels on exposure to the effluent blood of a perfused kidney, a connexion which can be established or refuted only by the identification of the agent responsible for each. Furthermore, the phenomenon with which this paper deals appears under highly abnormal conditions and the substitution of heparinized blood for defibrinated blood prohibits it. This last observation offered the opportunity of determining whether under these conditions ischæmia of the kidney causes the liberation of a substance producing constriction

of the vessels of the isolated loop of intestine. The reduction in renal blood-flow was produced by means of a screw-clip applied to the arterial supply tube, the reduced flows varying between 37·5 and 13·5 per cent. of the original rates, and we found that in none of the three experiments in which the point was tested, did intestinal vasoconstriction result from such reduction in the renal blood-flow while both kidney and gut were being perfused in parallel. When defibrinated blood is used as the perfusate the blood-flow through the kidney is large, and the production or liberation of the vasoconstrictor substance under these conditions may well be associated with the fact that the kidney so perfused becomes œdematous, a state which is not seen when heparinized blood is used instead.

As to the nature of the substance liberated when defibrinated blood is used as perfusate we have little evidence to offer. The liberation of the substance, its constrictor action on the blood-vessels of the perfused loop, and the development of refractoriness in them during their exposure to it, still occur in the presence of atropine. Our experiments also show that the substance is not rapidly inactivated or destroyed in defibrinated blood since it was still present in amounts sufficient to produce refractoriness in the gut-vessels in one experiment at least 24 minutes, and in another at least 45 minutes after the source of supply had been withdrawn. Further, the substance would seem to act on the sympathetic myoneural junctions or on the plain muscle of the vascular bed since degenerative section of the post-ganglionic sympathetic nerves leaves the response of the gut-vessels apparently unaffected. Although no data are available, so far as we are aware, on the reactions of denervated intestinal vessels to drugs, Burn's [1932] observations on the vascular responses of the perfused forelimb of the cat suggest that the agent liberated by the kidney is not of the tyramine-ephedrine type, since these compounds are inactive after degeneration of the post-ganglionic sympathetic supply to the limb. Whether or no the substance liberated by the kidney in our experiments is the same as that responsible for the pressor activity found by Tigerstedt and Bergman [1898] in saline extracts of the cortex of the rabbit's kidney we cannot say. Histamine is excluded by our finding that under the conditions of our experiments it produces vasodilatation in the perfused loop.

SUMMARY.

1. When a kidney of the dog was perfused with defibrinated blood by one heart-lung preparation and a loop of jejunum by another, the transposition of the kidney to the heart-lung-gut circuit was followed by a fall in perfusion flow through the gut. Similar but smaller

responses were observed when, in like experiments, a forelimb or a segment of large intestine was used in place of the loop of jejunum.

2. The kidney appeared to be specifically responsible for the phenomenon since it was absent in control experiments in which the spleen or the limbs of the dog were substituted for the kidney.

3. The kidney liberated the vasoconstrictor substance over long periods of perfusion (*e.g.* 3 to 5 hours) and the gut-vessels responded to it after periods of perfusion of similar duration.

4. A single exposure of the gut-vessels to the effluent blood from the kidney produced in them a refractoriness to subsequent exposures, and this persisted in spite of prolonged irrigation of the vessels with blood entirely free from the constrictor substance.

5. When the initial exposure was maintained, the constriction of the gut-vessels was followed by relaxation. This relaxation was shown to be due to the development of refractoriness to the substance liberated by the kidney.

6. The more quickly the intestinal vessels constricted on exposure to the effluent blood from the kidney, the more rapidly did the refractory state develop.

7. The liberation of the vasoconstrictor substance by the kidney and the refractoriness of the gut-vessels were observed in the presence of atropine in the perfusate in a concentration of 0·1 to 0·2 mg./100 c.c. blood.

8. The liberation of the substance and the refractoriness of the vessels also occurred when pump-lung or pump-oxygenator preparations were substituted for heart-lungs in the perfusion system.

9. The phenomenon of vasoconstriction in the gut when its vessels were exposed to the effluent blood from the kidney did not occur when, instead of defibrinated blood, the perfusate was blood rendered incoagulable by heparin. Nor under these conditions did it occur when the arterial supply to the kidney was severely obstructed. The addition of heparin to defibrinated blood did not, however, interfere with its occurrence nor with the subsequent development of a refractory state in the gut-vessels.

10. Degenerative section of the autonomic nerves around the superior mesenteric artery did not appreciably affect the responses of the jejunal vessels to the effluent blood from the kidney.

11. The renal substance was not rapidly inactivated or destroyed in the blood of the heart-lung preparation.

12. Histamine has been excluded from being the substance responsible for the observed constriction of the gut-vessels.

13. The results are discussed with a view to their possible bearing on experimental hypertension of renal origin.

It is a pleasure to thank Professor de Burgh Daly for his help in connexion with the technique of negative pressure ventilation used in some of our experi-

of the vessels of the isolated loop of intestine. The reduction in renal blood-flow was produced by means of a screw-clip applied to the arterial supply tube, the reduced flows varying between 37·5 and 13·5 per cent. of the original rates, and we found that in none of the three experiments in which the point was tested, did intestinal vasoconstriction result from such reduction in the renal blood-flow while both kidney and gut were being perfused in parallel. When defibrinated blood is used as the perfusate the blood-flow through the kidney is large, and the production or liberation of the vasoconstrictor substance under these conditions may well be associated with the fact that the kidney so perfused becomes œdematous, a state which is not seen when heparinized blood is used instead.

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Summary.

1. When a kidney of the dog was perfused with defibrinated blood by one heart-lung preparation and a loop of jejunum by another, the transposition of the kidney to the heart-lung-gut circuit was followed by a fall in perfusion flow through the gut. Similar but smaller

SOME OBSERVATIONS UPON SODIUM ALGINATE. By O. M. SOLANDT. From the Physiological Laboratory, Cambridge.

(*Received for publication 13th December 1940.*)

ALGINIC acid was discovered by Stanford in 1883 in the course of experiments on the extraction of iodine from Scottish kelp [Stanford, 1883]. It is now prepared commercially in the British Isles from the sea-weed *Laminaria hyperborea*. It is coming into general use as an ingredient of foods and to replace agar in many of its uses.

Alginic acid is probably a polymer of *d*-mannuronic acid with an empirical formula of $C_6H_{10}O_7$ and an equivalent weight of 194. The molecular weight varies with the degree of polymerisation from 40,000 to 250,000. The viscosity in solution is very high, and rises rapidly with increasing molecular weight. Alginic acid itself is unstable and only slightly soluble in water. The sodium salt is quite stable at room temperature and pH 5·5 to 8·5 and is soluble in water to the extent of 3 to 10 per cent. depending upon the molecular weight [Albright and Wilson, 1938; Rose, 1937; Nelson and Cretcher, 1929, 1930; Barry and Dillon, 1936; Dillon and McGuinness, 1931].

Sodium alginate is available under the trade name of Manucol in a variety of grades which differ in their average molecular weights. The range of molecular weights which is available includes the molecular weights of the plasma proteins. This suggested that sodium alginate might be a suitable substance for use in the treatment of shock when blood or plasma was not available for transfusion. Experiments soon showed that sodium alginate is toxic when injected intravenously and is quite unsuitable for this purpose.

THE TOXICITY OF SODIUM ALGINATE.

Sodium alginate solutions were tested for toxicity by intravenous injection into mice and rabbits. The tests were done with a 1 or 2 p.c. solution of Grade III sodium alginate (average molecular weight 85,000) in 0·9 p.c. saline. The minimum lethal dose was not determined accurately but is about 100 mg./kg. in rabbits. Four rabbits were given doses exceeding 125 mg./kg. and all died. Three were given a dose of 100 mg./kg.; one survived and two died. One rabbit received 85 mg./kg. and two 75 mg./kg. and all survived. The time of death varied from 15 minutes to about 15 hours after the injection.

ments. We would also express our indebtedness to the Government Grant Committee of the Royal Society for defraying part of the expenses incurred in this work.

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sedimentation of unwashed cells was similar or slightly more rapid. The increase of sedimentation rate in whole defibrinated blood was much the same.

Similar experiments were performed using whole heparinised blood from rabbits and rats. In both these animals the effect of sodium

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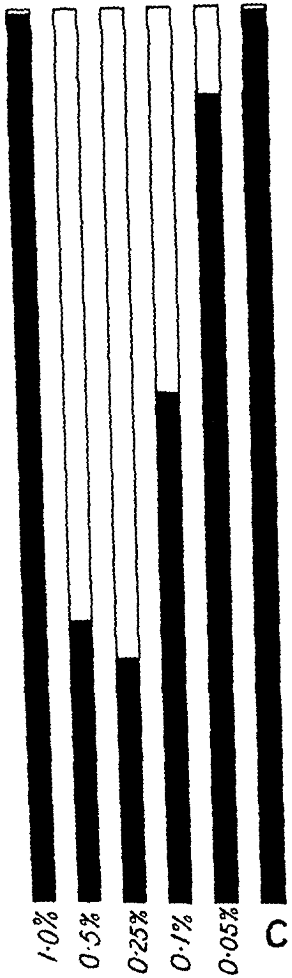

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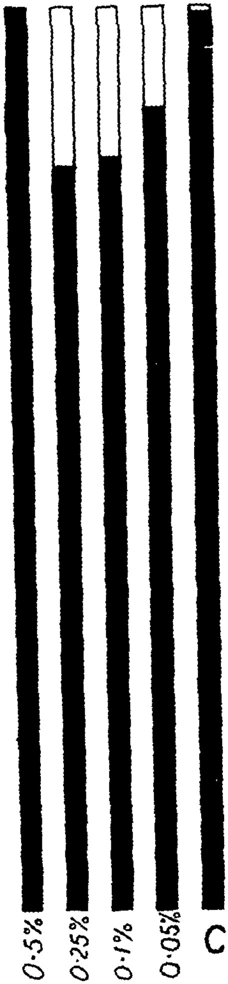

Fig. 1.—The sedimentation of washed human red cells in various strengths of Grade III (average molecular weight 85,000) sodium alginate solution in 30 minutes. (Average of four experiments.) C = control.

Fig. 2.—The sedimentation of washed human red cells in various strengths of Grade VIII (average molecular weight 185,000) sodium alginate solution in 30 minutes. C = control.

alginate upon the sedimentation rate in diluted whole blood is similar to that seen with human blood. Washed cells from the rabbit and the rat show a somewhat lower sedimentation rate with sodium alginate.

The earlier deaths were always accompanied by mild convulsions, marked cardiac slowing, and early cessation of respiration.

In mice (9) doses of 200–500 mg./kg. were fatal in from 1 minute to 12 hours.

In the rabbits, whether death was immediate or delayed, there was always marked dilatation of the right heart and often considerable pulmonary œdema. Sections of the lungs from three of these rabbits showed, in addition to the usual picture of pulmonary œdema, a very marked œdema of the adventitia of the arteries and arterioles. There was no similar lesion around the veins or bronchi. Sections of liver showed no abnormality. There was no gross or microscopic evidence of embolism due to precipitation of sodium alginate or to clumping of red blood cells.

The effect of the intravenous injection of sodium alginate on a lowered blood-pressure was tested in five cats under chloralose anæsthesia, the blood-pressure having been lowered by hæmorrhage. In all cases there was a transient rise in blood-pressure, followed by a fall which ended in the death of the animal if the dose of sodium alginate was large. The lethal dose varied greatly with the rate of injection. Doses between 120 and 450 mg./kg. were fatal within a few minutes. Marked slowing of the heart preceded death, but the heart continued to beat while the blood-pressure was falling and after respiration had ceased. At autopsy the right side of the heart was greatly distended and there was some pulmonary œdema.

No attempt was made to estimate the toxicity of sodium alginate when given by mouth or subcutaneously. It is widely used in food products and is apparently harmless.

The Effect of Sodium Alginate on the Sedimentation Rate of Red Blood Cells.

Sodium alginate greatly accelerates the sedimentation of red blood cells. Sedimentation rates were measured in Westergren tubes. Most of the experiments were done with a suspension of twice-washed red blood cells in 0·9 p.c. saline. A 40 p.c. suspension of cells was used, and this was diluted with an equal volume of the sodium alginate solution to be tested. In some cases whole blood was used and similarly diluted. The sodium alginate solutions were made up in normal saline so that all the solutions were isotonic or slightly hypertonic.

The results for normal, twice-washed, human red cells are shown in fig. 1. This is the average of four experiments. The extent of sedimentation in each of the various strengths of sodium alginate in 30 minutes is shown. In these experiments sodium alginate Grade III with an average molecular weight of 85,000 was used. Similar results were obtained with Grade I (average molecular weight 40,000). The

red cells into large aggregates. This clumping resembles true agglutination both macroscopically and microscopically but is readily distinguished by the ease with which the clumps can be shaken apart.

It seems reasonable to suppose that the slow sedimentation seen in the higher concentrations of sodium alginate is due to the high viscosity of these solutions. The clumping of the corpuscles is clearly visible even though they do not settle. Fig. 2 shows the sedimentation rate of washed human cells in Grade VIII sodium alginate (average molecular weight 185,000). The sedimentation rate is much lower than in Grade III, and the most rapid sedimentation occurs in a more dilute solution.

The effect of sodium alginate in producing an increased sedimentation rate cannot be due to its viscosity alone. If sodium alginate solution is heated to 100° C. it loses its viscosity before it loses its ability to produce an increase in sedimentation rate. This is shown in fig. 3. It is apparent that heating for 1 hour at 100° C., which causes an approximately tenfold reduction in viscosity, increases the sedimentation rate in 0·5 p.c. concentration and has very little effect on the sedimentation rate in 0·25 p.c. concentration. Heating for 4 hours produces a further decrease in viscosity and a marked diminution in the effect on the sedimentation rate.

Direct observation of the mesenteric vessels in the cat during fatal poisoning with sodium alginate sometimes showed clumping of corpuscles visible to the naked eye and sometimes did not. In three of the rabbits blood was drawn from the heart at death. The sedimentation rate of this blood (heparinised) was 3 mm. in 30 minutes. This is more than is normal in rabbits but is very slow compared to the rates seen *in vitro*. The amount of sodium alginate injected into these animals was sufficient to give an immediate concentration of 0·14 to 0·2 p.c. in the blood, assuming a blood volume of 70 c.c. per kilo.

Calcium and the Toxicity of Sodium Alginate.

Maas [1938] has recently shown that the immediate toxicity of some samples of gum acacia is due to the absorption of calcium from the blood by the sodium arabinate of which the gum is mainly composed. This is shown by the fact that the intravenous injection of calcium chloride prevents the lethal effect of such samples of gum and that calcium arabinate is non-toxic.

It seemed probable that a similar binding of the blood calcium might account for some of the toxicity of sodium alginate since the two substances have many properties in common. There is, however, the important difference that calcium arabinate is soluble, whereas calcium alginate is very insoluble.

In two rabbits the total serum calcium fell from 13·0 and 13·5 mg. per cent. to 11·2 and 11·8 mg. per cent. 1 hour after the injection of

In defibrinated horse blood, which normally has a very high sedimentation rate, 1 p.c. sodium alginate markedly diminished the

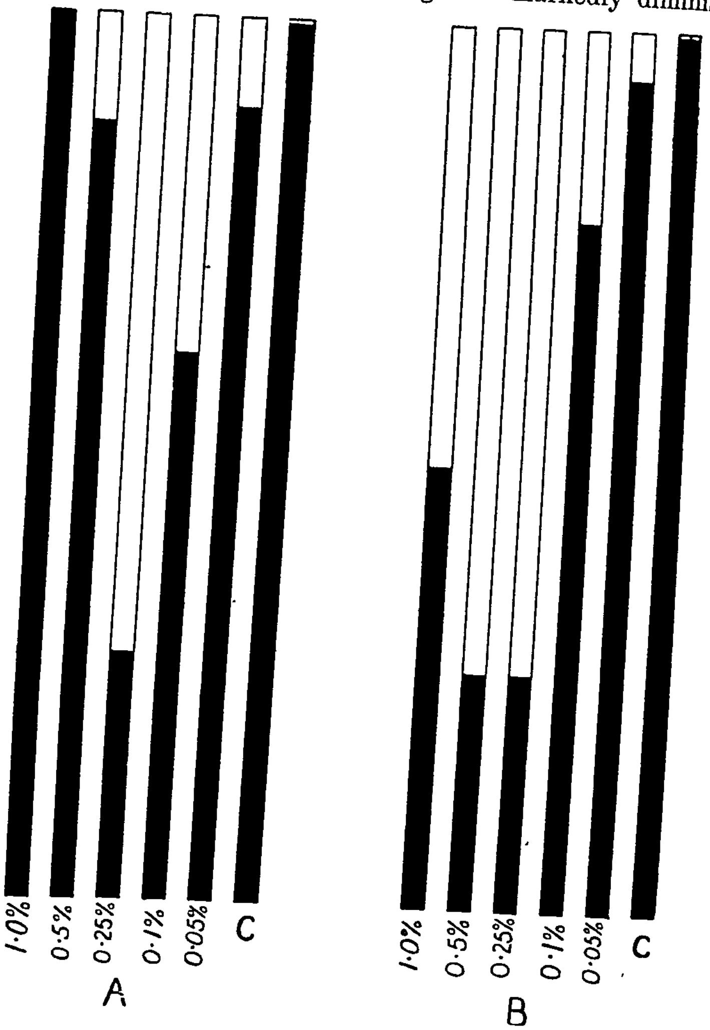

FIG. 3.

A. The sedimentation of washed human red cells in various strengths of Grade III sodium alginate solution. C = control.

B. The sedimentation of washed human red cells in the same solution after it had been heated for 1 hour at 100° C. The heating reduced the viscosity of the alginate solution approximately ten times and increased the sedimentation rate in the higher concentrations as shown. C = control.

sedimentation rate. Lower concentrations of sodium alginate increased the sedimentation rate just as in blood from other species.

Direct observation showed that the increased sedimentation rate in the presence of sodium alginate is due to the clumping together of the

INTERMEDIATE LOBE PITUITARY HORMONE. By F. W. LANDGREBE and H. WARING.

From the Department of Natural History, University of Aberdeen.

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CONTENTS.

I. INTRODUCTION.

A LARGE number of cold-blooded vertebrates belonging to the classes Pisces, Amphibia, and Reptilia exhibit a striking pigmentary response to changes of illumination, temperature, etc. Visible chromatic response in these forms results from the "expansion" and "contraction" of dermal and epidermal chromatophores. The most striking pigmentary change is the "background response," whereby animals darken when exposed to overhead illumination in surroundings which do not reflect light (*e.g.* black tank), and assume pallor in conditions of overhead illumination in surroundings which reflect or scatter light (*e.g.* white tank). The eye is the receptor for this response. In some forms, notably Amphibia, co-ordination of background responses is by means of blood-circulated pituitary hormones, and in others there is superimposed on this a direct nervous control of the melanophores. The evidence for pituitary hormonal control of the pigmentary effector system of certain fish and amphibia is well founded, although a fuller elucidation of some details is still called for. Hypophysectomised animals are paler than normal animals when exposed to overhead illumination in a black tank. Injections of pituitary extract evoke expansion of the melanophores of these operated animals. Extract of one frog pituitary contains sufficient excitant substance to darken *circa* 50 pale frogs [Hogben, 1924]. In one animal (*Anguilla*)

100 mg./kg. of sodium alginate. It is probable that the lowering of calcium would be better seen in an ultra-filtrate of serum, but the investigation was interrupted before this could be done.

In two rabbits a dose of 150 mg./kg. of sodium alginate was given and was followed within 10 minutes by the intravenous injection of 6 mg./kg. of calcium given as calcium chloride. Death was immediate in each case. Another rabbit was given 75 mg./kg. of sodium alginate intravenously, followed in 10 minutes by 5 mg./kg. calcium. There was an immediate convulsion, and death ensued in less than 5 minutes. Control experiments showed that doses of calcium up to 10 mg./kg. produced no obvious effect and that a dose of 75 mg./kg. of sodium alginate was not lethal.

Discussion.

The toxicity of sodium alginate when injected intravenously is much greater than would be expected from its chemical composition and physical properties. Its action in accelerating the sedimentation of red blood cells is very marked but does not appear to account for this toxicity. Other substances such as gum acacia have a similar effect on blood sedimentation but are much less toxic [Kruse, 1919, 1920; Lucia and Brown, 1934; Hanzlik and Karsner, 1919].

Sodium alginate may, like some samples of acacia, combine with calcium in the blood and lower the ionised calcium significantly. However, the toxicity of sodium alginate cannot be directly due to the lowering of blood calcium because the subsequent injection of calcium increases, rather than decreases, the toxicity of an injection of sodium alginate. It seems probable that the toxicity of sodium alginate is related to its precipitation as the insoluble calcium salt. There is, however, no evidence of such a precipitation in the microscopic sections of liver and lung from the injected animals.

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INTERMEDIATE LOBE PITUITARY HORMONE. By F. W. Landgrebe and H. Waring. From the Department of Natural History, University of Aberdeen.

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CONTENTS.

I. Introduction.

A large number of cold-blooded vertebrates belonging to the classes Pisces, Amphibia, and Reptilia exhibit a striking pigmentary response to changes of illumination, temperature, etc. Visible chromatic response in these forms results from the "expansion" and "contraction" of dermal and epidermal chromatophores. The most striking pigmentary change is the "background response," whereby animals darken when exposed to overhead illumination in surroundings which do not reflect light (*e.g.* black tank), and assume pallor in conditions of overhead illumination in surroundings which reflect or scatter light (*e.g.* white tank). The eye is the receptor for this response. In some forms, notably Amphibia, co-ordination of background responses is by means of blood-circulated pituitary hormones, and in others there is superimposed on this a direct nervous control of the melanophores. The evidence for pituitary hormonal control of the pigmentary effector system of certain fish and amphibia is well founded, although a fuller elucidation of some details is still called for. Hypophysectomised animals are paler than normal animals when exposed to overhead illumination in a black tank. Injections of pituitary extract evoke expansion of the melanophores of these operated animals. Extract of one frog pituitary contains sufficient excitant substance to darken *circa* 50 pale frogs [Hogben, 1924]. In one animal (*Anguilla*)

100 mg./kg. of sodium alginate. It is probable that the lowering of calcium would be better seen in an ultra-filtrate of serum, but the investigation was interrupted before this could be done.

In two rabbits a dose of 150 mg./kg. of sodium alginate was given and was followed within 10 minutes by the intravenous injection of 6 mg./kg. of calcium given as calcium chloride. Death was immediate in each case. Another rabbit was given 75 mg./kg. of sodium alginate intravenously, followed in 10 minutes by 5 mg./kg. calcium. There was an immediate convulsion, and death ensued in less than 5 minutes. Control experiments showed that doses of calcium up to 10 mg./kg. produced no obvious effect and that a dose of 75 mg./kg. of sodium alginate was not lethal.

Discussion.

The toxicity of sodium alginate when injected intravenously is much greater than would be expected from its chemical composition and physical properties. Its action in accelerating the sedimentation of red blood cells is very marked but does not appear to account for this toxicity. Other substances such as gum acacia have a similar effect on blood sedimentation but are much less toxic [Kruse, 1919, 1920; Lucia and Brown, 1934; Hanzlik and Karsner, 1919].

Sodium alginate may, like some samples of acacia, combine with calcium in the blood and lower the ionised calcium significantly. However, the toxicity of sodium alginate cannot be directly due to the lowering of blood calcium because the subsequent injection of calcium increases, rather than decreases, the toxicity of an injection of sodium alginate. It seems probable that the toxicity of sodium alginate is related to its precipitation as the insoluble calcium salt. There is, however, no evidence of such a precipitation in the microscopic sections of liver and lung from the injected animals.

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1935; Geiling and Lewis, 1935; Van Dyke, 1926; Fisher *et al.*, 1938]. Where an intermediate lobe exists, the presence of melanophore-expanding hormone in other parts of the gland is probably due to diffusion [Spaul, 1927].

In some forms there is no morphologically distinguishable intermediate lobe: fowl [de Beer, 1926], whale [Valso, 1934; Geiling, 1935], porpoise [Valso, 1932], and armadillo [Oldham, 1938]. In the fowl and whale the melanophore-affecting substance is located in the *pars glandularis* [Geiling, 1935; Kleinholz and Rahn, 1940].

There are then several possible sources for the extraction of B hormone, but now that pressor and oxytocic free extracts can be prepared from ox posterior lobes the others need not be considered.

Hitherto it has been customary to destroy the pressor oxytocic properties by treatment with caustic soda. It is pointed out subsequently that there are serious objections to this procedure.

III. Standardisation of Melanophore-expanding (B) Hormone.

Stehle [1936] and Teague *et al.* [1938] have recently expressed the need for a reliable method of assaying melanophore activity. A number of methods have been described, but as a preliminary to discussing their merits it is desirable to emphasise the requirements to be satisfied by any assay animal or method. These must be (1) specific power of the excitant substance to evoke the response which is to be measured, or failing that the limits of specificity must be known; (2) an objective measure of the response; (3) high sensitivity so that small amounts of the autacoid can be assayed; (4) reliability. Where it is necessary to choose between reliability and sensitivity the former is the more important, especially where, as in the present case of B hormone, the substance to be assayed lends itself readily to concentration.

Hogben and Winton [1922] were the first workers to attempt quantitative assay of B. They used graded doses injected into groups of normal (or hypophysectomised) frogs. Most subsequent workers have also used the frog [Abramowitz, 1937; Teague *et al.*, 1938; Teague, 1940]. With reference to the specificity of the reaction, Hogben and Winton [1922–23], Shen [1939], and Teague *et al.* [1938] have shown that a number of substances will evoke complete expansion of intact frog melanophores. Very few substances have any appreciable influence on the contracted melanophores of *hypophysectomised* animals [Hogben and Winton, 1922; Shen, 1937 *a* and *b*, 1939; Teague *et al.*, 1938]. The last authors, after a review of the literature, suggested that there is a species difference in this respect. They concluded from their own experiments on *Rana pipiens* that pituitrin is the only substance that will *fully* darken a hypophysectomised specimen. We have not

[Waring and Landgrebe, 1941] it has been possible to estimate with fair precision the amount of pituitary hormone circulating in the blood of dark animals. Recent work on the pigmentary effector system is summarised in Hogben [1924], Hogben and Slome [1936], Hogben and Landgrebe [1940], Parker [1936], Sand [1935], Abramowitz [1937, 1939], Kleinholz [1938], Neill [1940], and Waring [1936 *a* and *b*, 1938, 1940].

Pituitaries from vertebrates of all classes [Hogben, 1924] yield extracts which cause melanophore expansion of hypophysectomised Amphibia. In recent years attempts have been made to elucidate the rôle (if any) of the melanophore hormone in those vertebrates which have no melanophores. One necessary step in such investigations is the preparation of the substance in as pure a form as possible. This involves the separation of the principle from extraneous inactive protein and other pituitary autacoids without subjecting the crude substance to any chemical processing which may alter the molecule. Evidence has been advanced to show that the caustic soda treatment usually adopted to free an extract from pressor and oxytocic autacoids alters its melanophoric properties. The present paper describes a simple method for the preparation of melanophore or B hormone [Hogben and Slome, 1931] from the pituitaries of domestic food animals. The preparation is substantially free from pressor and oxytocic properties, and its high potency implies that it contains little extraneous protein. The extraction does not involve the use of alkalies or strong acids.

II. Source of Melanophore Hormone (B Hormone).

Crude extracts of either anterior or posterior lobe of the ox evoke melanophore expansion in pale hypophysectomised animals, but extracts of the latter have far greater potency [Hogben, 1924; Spaul, 1925–27; Van Dyke, 1936, 1940]. The ox posterior lobe is more accurately described as a neuro-intermediate lobe. It is made up of a neural lobe derived embryologically from the infundibulum which interdigitates with an intermediate lobe derived from the hypophysis, so that extraction of either tissue alone is for general purposes not a practical proposition. Unfractionated extracts of whole mammalian posterior lobe have several properties in addition to melanophore excitation, but there are several classes of evidence which show that the specific excitant of melanophores comes from the intermediate lobe, where this exists as a morphological entity. This evidence is derived from survival experiments after the separate removal of the various lobes, pathological and experimental destruction of the *pars intermedia*, the injection of extracts and work on implantation and tissue culture [Allen, 1916–30; Atwell, 1919–37; Smith, 1916–20; Smith and Smith, 1923; Swingle, 1921; Hogben and Winton, 1922; Hogben and Slome, 1936; Houssay and Ungar, 1924; Anderson and Haymaker,

systematic sensitivity experiments on the same animal before and after operation.

Where on grounds of expediency normals are used for assay purposes environmental conditions must be carefully standardised. It must be

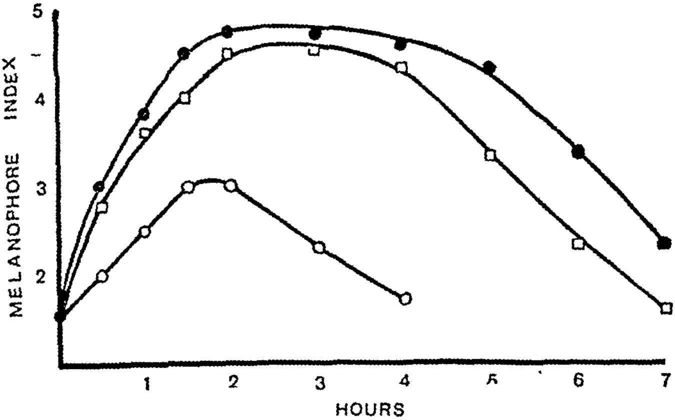

FIG. 1.—Increase of sensitivity after operation of completely hypophysectomised *Xenopus*. Responses of same group of animals to injection of 1 gamma carbon-extracted B. 15° C.

—○— 2 weeks after operation.
—□— 2 months after operation.
—●— 4 months after operation.

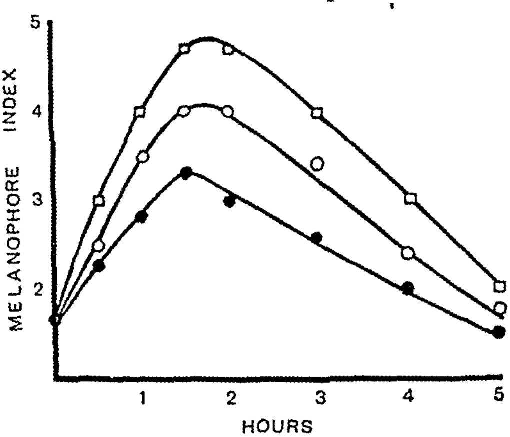

FIG. 2.—Responses of the same group of normal *Xenopus* to different doses of carbon-extracted B. White container with overhead illumination. 15° C.

All injections 1 c.c.

—●— $\frac{1}{4}$ gamma.
—○— $\frac{1}{2}$ gamma.
—□— 1 gamma.

realised, too, that there is a variation in sensitivity between different normals, and that they are never so sensitive as an animal hypophysectomised six months before use. In fig. 2 there is shown the

carried out similar experiments on *Xenopus*. Shen (private communication) informs us that he is conducting pharmacological investigations on *Xenopus*, and that so far he has found that this animal reacts similarly to *Rana*. On the other hand we cite an instance on p. 52 of a sample of urine in which the excitant substance was almost certainly not B evoking a full expansion of hypophysectomised *Xenopus* melanophores.

The sensitivity of the frog is high. The response can be accurately measured by taking readings of the melanophores in the posterior web. Hogben and Winton originally distinguished three grades of expansion. In 1930 Hogben and Gordon introduced an improvement on this and five arbitrarily chosen grades of melanophore expansion were recognised. By plotting the response against time two criteria for relative potency are obtained, so that a much higher order of discrimination can be reached between two samples than can be obtained by methods based upon the minimal dose.

The objection to the use of the hypophysectomised frog as a test animal is the difficulty of maintaining the animal alive for prolonged periods. Hogben and Gordon, in the paper referred to above, introduced *Xenopus* as a test animal and claimed to discriminate between doses of *circa* 20 per cent. difference. *Xenopus* can easily be maintained indefinitely in the laboratory by suitable feeding, etc. [Landgrebe, 1939], and can be bred, metamorphosed, and reared to adult state irrespective of the time of year [Landgrebe and Purser, unpublished]. Hogben and Slome [1931] showed that hypophysectomised *Xenopus* is much more sensitive to B hormone than the normal animal. The use of the hypophysectomised animal avoids any possibility that the injected substance stimulates endogenous secretion from the intact animal's own pituitary. We have now used hypophysectomised *Xenopus* as a test for B substance for some years in this laboratory. It fulfils all the requirements outlined above. It lives indefinitely, its response is not affected by lighting conditions within a wide range, estimations can be carried out at a water temperature (*e.g.* 15° C.), which is easily stabilised in the laboratory, and when once the animal has settled down after the operation it gives completely consistent responses to the same dose. The fact that it can be maintained for long periods provides an accurate cross check with standard dried extracts. The sensitivity of operated animals to B injections increases more or less consistently for approximately six months, after which the animal is completely reliable. The increase in sensitivity up to four months after operation is illustrated in fig. 1. At the end of six months after operation an injection containing $\frac{1}{4}$ gamma of the new carbon-extracted B will raise the melanophore index to 4. Comparison of the responses of normal and freshly hypophysectomised animals suggests that the former react to somewhat smaller doses, but we have not carried out

erythrophore potency. (*b*) Erythrophore hormone but not melanophore hormone is readily soluble in absolute ethanol; boiling sodium chloride solution extracts erythrophore hormone from posterior-lobe powder but leaves the melanophore hormone in the residue. (*c*) Extracts made from different parts of ox gland contained different proportions of the two properties; *pars intermedia* is richest in erythrosome-dispersing substance; basophil area richest in melanophore hormone.

Rodewald [1935] observed that pituitary extracts from frogs maintained in complete darkness evoked little response when injected into frogs, but caused erythrosome dispersion in *Phoxinus.*

We ourselves tested the effect of injecting large doses of the purified B hormone (*v. infra*) into *Phoxinus.* Two groups of five, including both males and females in each group, received 1 gamma and 10 gamma respectively. The erythrophores of one specimen of the first group reacted slightly and those of two specimens of the second group reacted definitely.

Zondek and Krohn [1932] were unable to detect erythrosome substance in the blood; they found it only in the third ventricle and the pituitary stalk: Lewis *et al.* [1937] could not confirm this. There is abundant evidence from other sources (Abramowitz, Waring, Parker, McLean, Krogh) to show that melanophore-expanding substance is easily detected in the circulation.

Apart from the general criticism that the isolated frog skin method used by Jores is unreliable, his findings in some cases are directly contrary to those of other workers. Jores himself and others have since claimed that melanophore hormone *is* soluble in absolute alcohol. The observation of Rodewald [1935], if true, may be purely a threshold phenomenon; but in any case the whole question of melanophoric properties of glands from dark-adapted frogs is at present in an unsatisfactory position (p. 44).

Further work may be expected to clarify the position.

When Zondek and Krohn [1932] introduced the *Phoxinus* test for chromatophoric pituitary substance, they claimed that it was preferable to the frog test because it was specific and the pH of the injected fluid did not influence the response evoked. If the emphasis on pH be taken to imply that amphibians are sensitive to small changes of pH of the injected fluid, it must be clearly emphasised that this is certainly not the case with hypophysectomised *Xenopus.* That Zondek's claim of specificity is unjustified has been shown by two subsequent workers [Collin and Drouet, 1914; Stutinsky, 1934]. They also showed that the response to injection in the female *Phoxinus* is dependent on the condition of the gonads.

Zondek's *Phoxinus* unit is based on the observation of macroscopic colour. In regard to melanophores, we have repeatedly emphasised

responses to different doses of B of normal animals selected for their high sensitivity and maintained under overhead illumination on a white background. It will be noted that normal animals more rapidly attain pallor after passing the peak of response than do hypophysectomised ones.

Hogben [1924] recorded that the melanophores of isolated segments of pale skin placed in Ringer containing pituitrin expanded. Physiologically this was an important observation in that it shows that the pituitary substance acts directly on the melanophore. It may also have an important application in the determination of the melanophore speed, but its value in the standardisation of pituitary extracts is doubtful, as the pH and ionic concentration of the fluid must be carefully standardised; there is a great difference in sensitivity between the skins from different animals at any one time, and there is a marked seasonal variation in sensitivity [Jores, 1933]. In addition, it shares with the perfusion method advocated by Fenn [1924] and McLean [1928] the fundamental disadvantage that the *same* test object cannot be used repeatedly. This method may have its uses as a qualitative test (if its sensitivity should be shown to be higher than that of hypophysectomised *Xenopus*), but we are sceptical of its value in quantitative work.

Kleinholz and Rahn [1940] have recently described a method for B assay using hypophysectomised *Anolis*. The advantage claimed is greater sensitivity than afforded by the hypophysectomised frog. These authors have adopted substantially the same routine as that suggested by Hogben in 1930 and described above. Their method suffers from two serious drawbacks: (1) estimation of the pigmentary response is based on macroscopic appearance, and (2) hypophysectomised *Anolis* does not live for long periods.

The only other method of assaying the chromatotropic pituitary hormone which has received much attention is the *Phoxinus* test described by Zondek and Krohn for their substance Intermedin. This was a pituitary preparation which evoked expansion of the melanophores of frogs and the erythrophores of *Phoxinus*. They did not suggest that these two effects might be dependent on two chemically different substances, and Böttger [1937] agreed that one and the same substance is responsible for both. If this is so, the *Phoxinus* assay appears to have no advantage over the amphibian test, which is obviously more convenient.

There is, however, a body of data which, if reliable, definitely implies the existence of two distinct activators. The evidence advanced by Jores [Jores and Lenssen, 1933; Jores and Will, 1934] may be summarised as follows: (*a*) Activation of erythrophore excitant is brought about by acid; activation of melanophore hormone by alkali; boiling with alkali increases melanophore potency and decreases

erythrophore potency. (*b*) Erythrophore hormone but not melanophore hormone is readily soluble in absolute ethanol; boiling sodium chloride solution extracts erythrophore hormone from posterior-lobe powder but leaves the melanophore hormone in the residue. (*c*) Extracts made from different parts of ox gland contained different proportions of the two properties; *pars intermedia* is richest in erythrosome-dispersing substance; basophil area richest in melanophore hormone.

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Zondek's *Phoxinus* unit is based on the observation of macroscopic colour. In regard to melanophores, we have repeatedly emphasised

that macroscopic matching of colours is in itself difficult and, owing to individual variations in the concentration of effector units, is inevitably open, even with skilled matching, to a high degree of error. Böttger [1937] has described a method of microscopically examining erythrophore expansion which may eliminate this pitfall.

Even if further investigation should make it clear that two distinct excitant substances do not exist, we see little reason for continuing with the *Phoxinus* erythrophore test. The disadvantages of the method are evident. If there prove to be two excitant substances, then some modification of the method must be evolved whereby erythrophore indices can be assessed like those of the melanophores in the amphibian web.

IV. Methods of preparing B-containing Extracts from Ox Gland substantially free of Pressor and Oxytocic Properties.

(a) *Previous Methods.*

Methods for the preparation of B-containing extracts of pituitary gland have been described by Hogben and Gordon [1930], Zondek and Krohn [1932], Stehle [1936], Teague [1939], and Fostvedt [1940].

All these procedures are lengthy and, with the exception of Zondek's product prepared from anterior lobe (which necessarily has a low yield), *they all require treatment with alkali to remove pressor and oxytocic autacoids.* Dietel [1934] has described the preparation of a very potent extract, but here the initial extraction involves the use of alkali. The method described below utilises the richest source of the substance (posterior lobe), is rapid, necessitates no drastic chemical treatment, and yields a product substantially free from oxytocic and pressor properties.

(b) *New Carbon-extraction Method.*

The process is a very rapid one, and the final product can be obtained from the original powder in under three hours. In some cases we used freshly excised glands which were immersed in acetone immediately after separation of the lobes and subsequently powdered. Equally good results were obtained by the use of an acetone-dried posterior-lobe powder produced commercially by the firm of Oxo, Ltd.

5 g. ox posterior lobe pituitary powder were boiled for 15 minutes with 25 c.c. distilled water. The extract was then filtered while still hot on a Büchner funnel and the powder re-extracted with 25 c.c. hot water and filtered. The filtrates were combined and yielded about 45 c.c. of extract. 2 g. of freshly activated animal charcoal were added to the extract and left for 30 minutes with occasional stirring. The extract was filtered on a Büchner funnel until the filtrate was free from charcoal. The charcoal was then washed on the funnel with water, dried and extracted for 1 hour with 20 c.c. of pure phenol at

50° C. No melanophore hormone could be detected in the filtrate in doses of 1 c.c. The phenol extract was filtered in the incubator and the filtrate, while still warm, was poured into 100 c.c. of acetone, when the active principle was precipitated. If a few drops of concentrated HCl are added, the precipitate is thrown down more quickly and a higher yield results. After 10 minutes the extract was centrifuged at about 1000 r.p.m. and the supernatant fluid discarded. The precipitate in the bottom of the tube was washed twice with 50 c.c. acetone and allowed to dry. When dry the precipitate was weighed, the yield usually being about 25 mg.

For general purposes we dissolve 1 mg. in 10 c.c. hot water. This solution (100 γ/c.c.) when boiled, sealed, and kept in the dark retains its potency for at least one month. We have not experienced any need to keep solutions for longer periods. Appropriate dilutions are made prior to each injection.

Using both hypophysectomised and normal *Xenopus* for test purposes, we have consistently found the same response to the same weighed quantity of powder even 6 months after extraction. We therefore believe that the dried powder kept in a desiccator retains its potency indefinitely.

If this is so, a convenient melanophore standard could be used, based on weighed quantities of the powder. For example, we have found ¼ gamma injected into a fully "matured" hypophysectomised *Xenopus* (30 g.) will evoke expansion of its melanophores to $\mu=4$. We do not feel, however, that the time is yet opportune to suggest a standard of melanophore activity based on either weighed quantities of powder or on the response of test animals. While there is reason to believe that the product described approaches more closely to a pure autacoid than any previously described, attempts at even further purification are in progress.

Further, we have grounds for hesitancy in attempting the standardisation of any chromatophoric pituitary product in terms of weight of dry powder, since in our experience extracts made according to Hogben and Gordon's method have yielded at different times of the year products of widely different potencies. This implies that the weight of glandular material used in the first place has no necessary relation to the amount of melanophore-expanding substance in the final solution. The variation in B content of the fresh gland may be due to lighting condition, season [*cf.* Waring, 1936 *b*], or other unassessable influences. Unless the method of extraction specifically isolates the B hormone, it is evident that final products isolated from different samples of gland may well vary in their activity per unit weight. We have prepared our powder from both pig and ox posterior lobes collected at various times of the year, and also from "Oxo" dried posterior lobe. The yield of powder from the same weight of

original material (25 g. fresh issue) varies considerably (18–52 mg.). At the same time the activity of unit weight of the final product from these various sources remains approximately constant.

In general, an objection to the biological standardisation of melanophoric substance lies in the fact that different extracts may evoke a different kind of response (*cf.* p. 42) as distinct from different degrees of darkening. Until we have more definite information in regard to the effect of pressor and hypothetical precursor hormones, etc., on the response we cannot formulate a scheme which distinguishes between two samples which, although causing the same rise in index, maintain the expanded condition of the melanophores for quite different periods. As, however, it is desirable to have some unit for comparison, we adopt for the present ¼ gamma of our powder as a unit. *¼ gamma* (0·00025 mg.) *will raise the melanophore index of a completely hypophysectomised* Xenopus *to* 4·0 (*i.e.* a submaximal response).

Properties.

The final powder is a brown powder of high potency. Its potency implies a high degree of purification, but nitrogen tests for protein are positive. This may mean either that the product is contaminated with extraneous protein, or that the active principle is itself a protein or closely bound to a protein complex. Zondek and Krohn [1932] claim that Intermedin is protein free, but the tests applied may not have been exhaustive enough. Quite apart from the high potency of our product the following observation furnishes suggestive evidence of its homogeneity. An extract was made from 2 g. of acetone dried pig posterior lobe according to the above method. The yield (6 mg.) was much lower than that obtained from an equivalent weight of ox gland, but 1 gamma produced almost identical response curves in hypophysectomised *Xenopus* as 1 gamma of the powder prepared from ox pituitaries. Yields obtained from anterior lobe ox have similar properties.

Our product is readily soluble in hot water and up to fairly high concentration in cold water. Four volumes of acetone added to one volume of an aqueous extract precipitates the active substance. It is only slightly soluble in cold absolute ethanol and fairly soluble in hot absolute ethanol. It is insoluble in acetone and ether.

Although there is no generally accepted method for the assay of B hormone, we believe that no other product derived from posterior lobe has been obtained with such a high B content and such slight pressor and oxytocic properties. Indirect evidence derived from the effect on caustic treatment (p. 48) points to the same conclusion. It is most closely approached by a preparation by Stehle [1936], which is active in doses of ·001 mg. judged by its effect on the melanophores,

and contains "less than ½ unit of pressor per 100 melanophore units." A melanophore unit is not defined, but presumably it is the quantity necessary to darken completely one pale frog.

The product is virtually free from pressor and oxytocic properties. Pressor activity was determined by measurement of the carotid blood-pressure of the anæsthetised cat (fig. 3). It is doubtful whether any

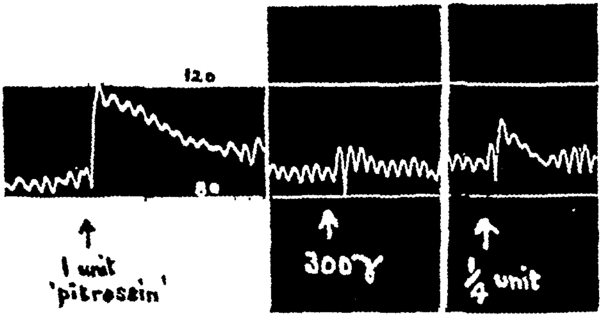

FIG. 3.—Dial anæsthetised Cat. Heparin. Both vagi severed. Carotid blood-pressure.

A. 1 International pressor Unit.
B. 300 gamma carbon-extracted B.
C. ¼ International pressor Unit.

All injections 1 c.c., followed by 1 c.c. saline.

significant rise in blood-pressure was ever evoked. Certainly doses up to 1000 gamma contained less than 1 International Unit. Oxytocic activity measured on the uterus of virgin pigs showed less than 1 International Unit in 10,000 gamma.

V. The Physiological Properties of Posterior-lobe Extracts after Treatment with Charcoal.

The fact that our preparation from the posterior lobe exhibits little or no pressor (less than 1 unit per 1000 gamma), oxytocic (less than 1 unit per 10,000 gamma), or anti-diuretic properties may mean that under the conditions of our technique the substances responsible for these properties are not adsorbed on the charcoal or that they are not eluted by phenol.

As a preliminary to work now in hand, some tests were made with the extract before and after charcoal treatment. The crude extract was assayed for melanophore and pressor activity. It was found that 1 c.c. containing the equivalent of 0·04 mg. of original posterior-lobe powder produced the same response in hypophysectomised *Xenopus* as 1 gamma of our final powder. The equivalent of 1 mg. of the original powder was found to contain about 2 pressor units, and it

was observed that the rise in blood-pressure in the anæsthetised cat was preceded by a slight fall—indicating that histamine was present. Our product, therefore, is 40 times as potent in B hormone as the original powder, and *at least* 98 per cent. of the pressor activity has been separated from the melanophore hormone. Stehle's [1936] final product was 25 times as active as his original powder.

After charcoal treatment the filtrate showed no melanophore activity, and gave a depressor response with no subsequent rise in blood-pressure when 1 c.c. (0·05 g. original powder) was injected into the anæsthetised cat. This solution was also assayed for oxytocic activity, and gave a response equivalent to 4·5 International Units per c.c. (0·05 g. original powder). This response was probably due to the presence of histamine. In any case it means that most, if not all, of the oxytocic activity has been adsorbed on to the charcoal. This confirms the finding of Gulland and Newton [1932]. These facts indicate that very little pressor and practically no oxytocic activity is eluted by phenol in 1 hour.

Only a fraction (usually about 25 p.c.) of the B hormone in the original powder is extracted by our technique, but in view of the large quantities of the hormone available this is only a minor disadvantage. As all the B hormone is removable from the crude extract by charcoal, it may be that increased yield would result from eluting with phenol for a longer time, but it is probable that such a procedure would result in an increase in the amount of pressor activity in the final product. This point is being investigated further.

VI. The Response of *Xenopus* to various B-containing Extracts.

There are two well-authenticated results of treating posterior lobe extracts with caustic soda: (*a*) a greatly increased melanophore-expanding potency; (*b*) a pronounced increase in the duration of the response *when judged on submaximal responses* (fig. 4) [Hogben and Gordon, 1930; Jores *et al.*, 1933–34; Stehle, 1936; Böttger, 1937].

On the data hitherto presented in the literature these two effects of caustic soda treatment of pituitary extracts may affect their melanophoric properties in at least four ways:

(*a*) By acting on some constituent of the extract with no melanophoric properties to form B, so that there is an absolute increase of B.

(*b*) By modification of the extract so that the hormone is less easily excreted or destroyed.

(*c*) By destruction of the pressor autacoid so that the availability of B to the tissues is increased.

(*d*) By modification of the B molecule itself so that
(1) the potency of the extract is *increased*, and
(2) the rate of excretion or destruction of the autacoid is *reduced*.

It is convenient to consider these separately.

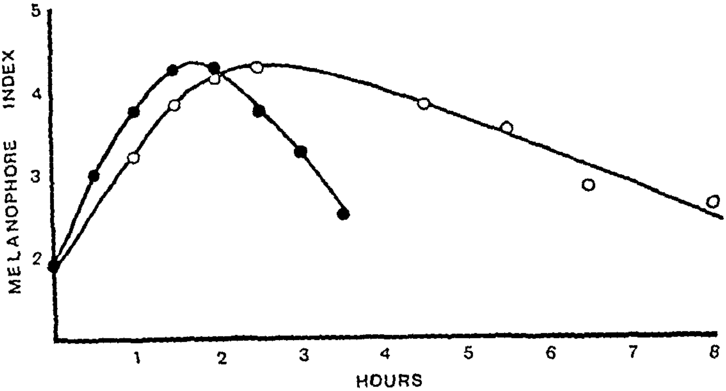

FIG. 4.—Completely hypophysectomised *Xenopus*. Responses to ox posterior-lobe extracts.

All injections 1 c.c. 15° C.

—●— untreated aqueous extract.
—○— same extract boiled with caustic soda. ½ the above dose.

(a) *Absolute Increase in B.*

The view that caustic soda treatment increases the melanophore potency of pituitary extracts by causing an absolute increase of B is based chiefly on experiments with pituitaries removed from animals kept in darkness. Evidence of different workers is contradictory. Koller and Rodewald [1933] claimed that extracts of pituitaries from frogs maintained in complete darkness have a lower melanophore-expanding potency than similar extracts prepared from frogs maintained under constant illumination. Jores [Jores, 1934; Jores and Will, 1934; Jores and Hoeltz, 1936] confirmed this result using both saline and weak acid for extraction, but claimed that the difference between the two extracts was eliminated by the use of caustic soda extraction. He interpreted this in the following way. In darkness the precursor to B is present in the pituitary but very little effective B. Caustic soda acts on the precursor to produce definitive B. In a more recent paper Rodewald [1935] has been unable to confirm Jores's findings. It is clear, of course, that Jores's findings could equally well be interpreted in terms of the destruction of an inhibitive substance by caustic soda.

Kleinholz and Rahn [1940] found no significant difference between caustic soda extracts of pituitaries from animals maintained in darkness and under constant illumination. They did not make comparable

extracts using neutral saline or weak acid. Masselin [1939] found that pituitaries from *Bufo* kept in darkness for 14 days showed an *increase* in B content but that shorter periods in darkness were without effect. His experiments with different methods of extraction led him to conclude that alkaline, acid, or saline extracts of pituitaries from animals kept under like conditions were equal in their B content.

In the face of such conflicting testimony it is impossible to draw any conclusion. Two things may be noted, however: (*a*) the contradictory results are probably due in large measure to unreliable assay methods; (*b*) that even if there is a B-precursor in the gland under certain circumstances which can be activated by caustic soda, this can only explain the increased expanding power and not the flattened time-effect curve. To explain the latter some other effect of the treatment must be invoked, *e.g.* modification of the rate at which the substance injected is excreted or destroyed. Alternatively it would be necessary to postulate that the newly formed B has properties different from the substance normally present in aqueous extracts of posterior lobe.

(b) *Modification of Excretion and/or Destruction Rate of B.*

Whether B is ultimately excreted or is destroyed by the tissues is not known. What little evidence exists points to the latter (p. 50). If, however, B is passed by the kidney, the presence of renal regulators in the injected fluid would be expected to have an important effect on the shape of the response curve and perhaps also on the degree of melanophore expansion.

Posterior-lobe extracts injected into unæsthetised animals inhibit the flow of urine. Heller [1939–40] has shown that inhibition of diuresis is due to a substance present in pressor fractions but separable from pressor by its heat resistance. Using Burn's [1931] apparatus for collecting urine, we were unable to detect any anti-diuretic effect in caustic-treated (*i.e.* pressor-free) extracts injected into mice. If renal activity enters into these considerations at all, we should expect, in the absence of evidence to the contrary, that anti-diuretic activity would tend to prolong responses. So if B is excreted by the kidney, treatment of the extract with caustic soda would have the opposite effect on the time-effect curve to that we obtained (fig. 4).

(c) *Destruction of the Pressor Component.*

When Hogben and Gordon [1930] discovered that caustic soda not only destroyed the pressor activity of extracts but augmented their melanophore activity, they attributed the latter effect to the increased availability of B to the tissues. Fig. 5 shows that extracts containing as little as $\frac{1}{250}$ of a pressor unit evoke responses significantly different from caustic soda-treated pressor-free extracts. Should this

amount appear insufficient to have a significant effect on the peripheral circulation, the following citation [Hogben, 1927] confirms that extremely small doses evoke constriction: "Krogh has shown that

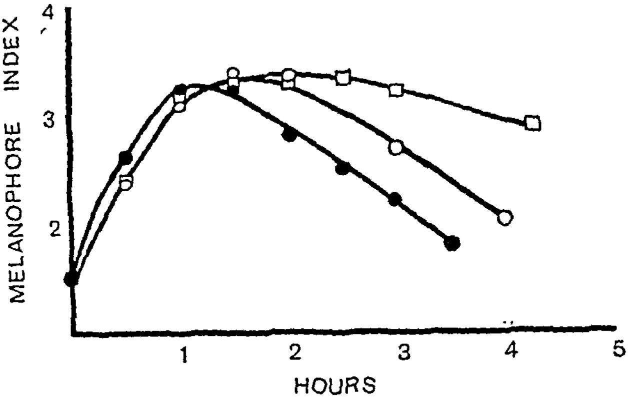

FIG. 5.—Completely hypophysectomised *Xenopus*. To show the duration of response evoked by three different B-containing extracts in doses that evoke the same rise of index.

All injections 1 c.c. 15° C.

—●— Pitressin containing $\frac{1}{250}$ International pressor Units.

—○— carbon-extracted B.

—□— whole posterior lobe after treatment with caustic soda.

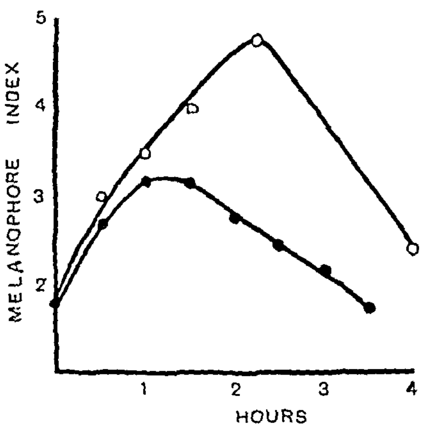

FIG. 6.—Completely hypophysectomised *Xenopus*.

All injections 1 c.c. 15° C.

—○— containing $\frac{1}{60}$ pressor units.

—●— containing $\frac{1}{500}$ pressor units.

visible effects on the capillaries alone are produced when the vessels are perfused with a Ringer solution containing a concentration of one in a million commercial pituitary preparation. Assuming the latter to be a 10 per cent. extract, this represents a dilution of 1 : 10,000,000 fresh glandular substance."

Although subsequent investigation has shown that the two effects of caustic treatment under consideration are not due to the destruction of pressor substance, two things need emphasis:

(*a*) Pressor substance in B extracts does have a very marked effect on the distribution of B as judged by reading the web of *Xenopus* at different locations. Also large doses of Pitressin evoke a macroscopically apparent patchwork of light and dark areas in *Scyllium* [Waring, 1936].

(*b*) Fig. 6 shows that an increase of pressor substance, together with a comparable increase of B, leads to a sharper fall in the melanophore index once the response has passed its peak.

(d) *Modification of the B Molecule.*

Jores *et al.* [Jores and Lenssen, 1933; Jores and Will, 1934], Böttger [1937], and Stehle [1936, 1938] concluded that caustic soda treatment modified the B molecule itself. Stehle clearly envisaged the problem in the following way. The two effects observed after treatment may be due to destruction of the pressor autacoid or to modification of the B molecule. He accepted the second interpretation, since caustic treatment of his own B preparation (which has a very low pressor content) still brought about the characteristic effects.

It might be anticipated that some light would be shed on these several issues by observing the effect of caustic soda on (*a*) B extract obtained from naturally occurring sources which has no pressor activity; (*b*) B extracted by carbon from ordinary sources which is also substantially free of pressor activity.

(a) *Effect of Caustic Soda on the Responses evoked by naturally occurring Pressor-free B.*

The number of glands which contain B with little or no pressor activity are limited. Some of these are listed on p. 33. For our present purpose we selected the posterior lobe of the dogfish. It is not definitely known that this gland does not contain *any* pressor substance, but the large dose of 50 mg. dried powder evokes no response in the spinal cat [Hogben and de Beer, 1925]. The pressor content is therefore at the best only $\frac{1}{100}$ of the ox gland powder used in these investigations.

Dogfish glands were removed from anæsthetised fish which had been maintained for at least a month prior to operation in an illuminated tank in order to eliminate from the gland any precursor which may form in glands of animals in darkness (p. 43). As soon as they were extirpated the glands were placed in excess pure acetone which was changed before storage. 10 mg. of dry powder (4 glands approx.) were boiled for 15 minutes on a water-bath. After filtration the filtrate

(22 c.c.) was accurately divided into two parts. Normal caustic soda was added to both parts to N/10. One was immediately neutralised with HCl. The other was heated at 100° C. for 10 minutes and then neutralised. Both samples were boiled and sealed in tubes. Fig. 7 shows the responses of the same group of hypophysectomised *Xenopus* to injections of these extracts. The data show four things quite clearly: (1) Extract boiled with caustic soda is four times as potent as the untreated extract as measured by the *height* of the response curve; (2) untreated dogfish extract evokes a similar type of response

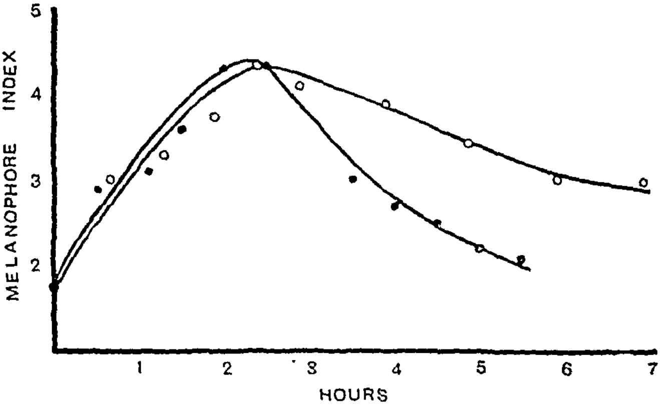

Fig. 7.—Completely hypophysectomised *Xenopus*. Responses to Dogfish posterior-lobe extracts.
All injections 1 c.c. 15° C.
—●— untreated aqueous extract.
—○— same extract boiled with caustic soda. ¼ the above dose.

to that evoked by untreated ox posterior-lobe extract; (3) extracts treated with caustic soda, like caustic-treated ox extracts, show a much prolonged response; (4) the melanophore index increases more rapidly after injection of untreated extract. In this respect it likewise resembles ox material.

Before drawing any detailed conclusions from these data we made comparable experiments with ox posterior-lobe powder.

A sample not boiled with caustic was assayed for pressor content using a Dial anæsthetised cat with both vagi severed. The equivalent of 1 mg. of original powder was found to contain approximately 2 International pressor Units. The melanophoric properties of the two extracts were assayed on the same group of hypophysectomised *Xenopus* previously used. The responses are shown in fig. 4. As judged by the melanophore-expanding potency (as distinct from the shape of the time-effect curve), the caustic-treated sample is twice as strong as the untreated sample.

Taken together, the [illegible] nd 7 permit us to

conclude (1) *that the degree of potentiation wrought by caustic treatment is not directly proportional to the pressor content of the extract before treatment*; (2) increased duration of the response after caustic treatment is evinced equally by extracts which previously had a high pressor content (ox) and extracts with little, if any, pressor properties (dogfish).

(b) *Effect of Caustic Soda on the Responses evoked by Carbon-extracted B.*

The B-containing extract described in this paper (p. 41) probably contains a lower pressor content than any previous preparation derived from mammalian posterior lobe prepared without caustic treatment. If the increased expanding potency and/or the flattened time-effect curve evoked after caustic treatment of general posterior-lobe extracts are due to the destruction of pressor activity itself, then we should expect that injections of our preparation would evoke a response not dissimilar from that evoked by general extracts after caustic treatment. *Actually injection in small doses evokes a response more closely resembling that of extracts rich in pressor activity* (fig. 5); *caustic soda* (*cold or hot*) *has no effect on expanding potency or duration of response.*

From (*b*) we can conclude that the two effects of caustic treatment are not due *primarily* to the destruction of pressor activity as such. Nor are they due to alteration of the B molecule itself unless we are prepared to make the unlikely assumption that adsorption on charcoal or its subsequent elution with phenol renders the molecule resistant. From (*a*) we can conclude that the effects of caustic treatment are not due to changes which can only take place when pressor substance is present prior to treatment. The fact that the two effects were produced by caustic soda treatment of pituitaries removed from fish maintained in illuminated tanks rules out a B precursor which can be activated by alkali such as that postulated by Jores.

There is sufficient evidence (p. 46) to show that *large amounts of pressor substance* inevitably affect the response to B. Exact information on the significance of pressor activity in melanophore responses will only be known when there is available a B-free pressor extract which can be used with pressor-free B in known amounts. We are aware of only two claims for the preparation from ox posterior lobe of a pressor extract free from B [MacArthur, 1931; Stehle and Fraser, 1935]. We carefully repeated MacArthur's prescription, but are unable to agree with him that the final product has no melanophoric properties. We are now attempting to prepare a B-free pressor extract. The fact that caustic soda treatment of our carbon extract does not alter its melanophoric properties shows that the very small amount of pressor substance in this extract does not have a measurable effect.

In the meantime, we should like to draw attention to the following

facts which appear to indicate that pressor substance present in the quantities normally injected with general posterior-lobe extracts does have a significant effect on the speed at which pallor is resumed after injection. The drop in melanophore index after a dose of our new preparation which evokes a rise to just over 3, is at approximately the same rate as one which evokes a rise to nearly 5 (fig. 8). On the other hand, comparison of the time-graphs in figs. 6 and 8 shows that this is not the case with pressor-containing extracts. This sharper fall in the

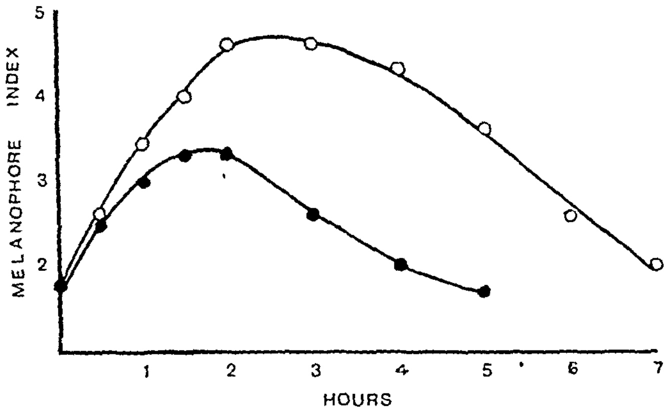

FIG. 8.—Completely hypophysectomised *Xenopus*. Responses to two different doses of carbon-extracted B. The doses were selected by trial to give peak points comparable with those in fig. 6.

All injections 1 c.c. 15° C.

latter may be due to pressor substance or to some other substance present in Pitressin. There is at present no evidence for the latter.

Following consideration of (*a*) an alteration of the B molecule, (*b*) the destruction of pressor substance itself or some change which only takes place in the presence of pressor substance, (*c*) the activation of a B-precursor as the prime cause of the two effects evoked by caustic treatment of general extracts, experiments of another kind were undertaken.

Working on the assumption that there is some component in general extracts other than vasopressin or a B-precursor which when treated with alkali brings about the changes in potency and duration of response under consideration, we dissolved carbon-extracted B in the B-free filtrate from which it had been separated and boiled it with caustic soda. No change in melanophoric properties resulted. The reason for this was apparent when we examined a second alternative.

A general ox posterior-lobe extract was boiled with N/10 caustic soda. Carbon was added and the adsorbate eluted with phenol. Injections of this extract evoked a response similar to that of caustic-treated general extract (*i.e.* flattened time-effect graph). We attempted

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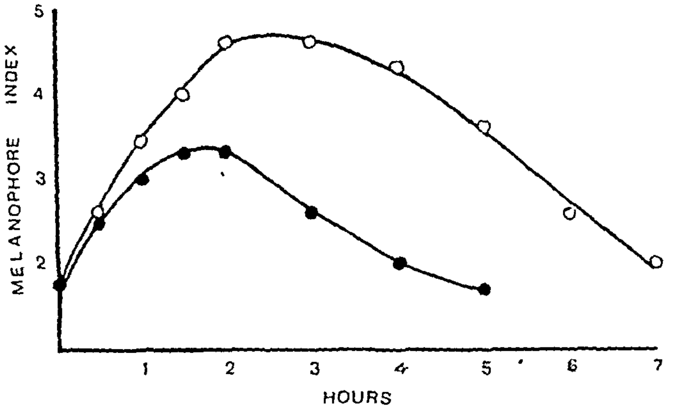

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quantitative experiments to determine whether carbon extracts from caustic-treated extracts also had increased expanding power, but, owing to the uncertainty as to whether all the active substance is precipitated in apparently comparable experiments, we are at the moment unwilling to draw any definite conclusion.

If the conclusions drawn on p. 48 are well founded, then from these last data the following conclusions are permissible:—

(*a*) A substance (or substances) other than vasopressin or Jores's B-precursor is present in general posterior-lobe extracts, which, *after treatment with alkali*, modifies the melanophore response evoked by B in at least two ways.

(*b*) The substance is adsorbed on to charcoal but is not eluted by phenol. This follows from the fact that caustic treatment of carbon-extracted B alone, or B dissolved in the filtrate from which it was separated, has no effect on the melanophore response.

(*c*) After treatment with caustic soda the substance responsible for the increased duration of response is adsorbed on to charcoal and eluted by phenol, together with B itself (p. 49).

Inhibition of B excretion or reduction in the rate of its destruction by the tissues would explain the longer duration of the response and possibly also the higher melanophore index evoked. While the evidence (p. 53) against the renal excretion of B is not sufficient to warrant any definite conclusion, we are at present more disposed to regard the effects noted as being due to destruction of B in the tissues.

An alternative to postulating the existence of a substance which when alkali treated has the two effects noted, is the hypothesis that "pure" B evokes a response of long duration and there exists in general extracts some substance other than vasopressin which evokes the "steep" recovery. This is untenable, because we should have to postulate that this substance is adsorbed on to charcoal. Were this so, we should obtain a flattening of the response curve after treatment of carbon-extracted B with caustic soda.

The substance in question may be oxytocin. Hogben and Gordon [1930] recorded that caustic soda treatment of Parke Davis Pitressin increased its melanophoric properties. Judging from the amounts of Pitressin injected by Gaddum [1928], Hogben and Gordon [1930], and Hogben and Slome [1931] compared with those injected by us, this product has changed its properties considerably during the intervening time. We boiled samples of Pitressin with N/10 caustic soda and found no increase in potency and no greater duration of response than would be explained by the destruction of the pressor properties.

VII. The Fate of B Hormone in the Animal Body.

Recent work [McLean, 1928; Waring, 1936, 1938; Waring and Landgrebe, 1941; Parker, 1936; Abramowitz, 1937; Jores, 1936] has shown that B hormone can be detected in the blood.

Evidence as to whether it is destroyed by the tissues or is excreted is less certain. Zondek and Krohn [1932] were unable to detect B in urine. Data suggesting that it is excreted in the urine come mainly from clinical sources. Van Dyke [1940] in a recent review has stated that it can be detected in the urine of normal men and women. Collin and Drouet [1933] claimed that urine from normal women the day before or on the first day of the menses showed an increased quantity of B hormone. They also detected an increased quantity in the urine of certain pathological cases in which the pituitary was believed to be involved (migraine, pituitary tumours, hyperthyroidism, retinal bleeding). Numerous authors have claimed an increase during pregnancy [Mandelstamm, 1935; Jores, 1936; Bruckmann, 1937], and some have even considered that it could be used as a basis for a reliable pregnancy test [*e.g.* Dychno, 1936]. This is, however, denied by most workers.

Cunningham-Dax [1938] presented a report on 20 cases of *retinitis pigmentosa*. Urine was made alkaline to litmus, boiled and stored in the dark. 0·3 to 1 c.c. were injected into normal pale or decerebrate frogs. In some cases isolated skin was immersed in the solutions suspected of containing B hormone. All 20 samples evoked expansion of the melanophores. Positives were sometimes obtained with isolated skin which, when injected into whole animals, evoked no response. Cunningham-Dax also confirmed Collin and Drouet's observation that there is an increase of melanophore stimulant in the urine at the beginning of the menstrual period. He obtained negative results from the urine of normal people, save under the following circumstances. If 3 c.c. instead of 1 c.c. were injected *or* if the urine was made alkaline by addition of 5N caustic soda instead of N/10 caustic soda, then positive results were sometimes obtained. We have little confidence in these findings, for the reasons previously stated on p. 36. If decerebrate implies the *complete* elimination of endogenous pituitary influence, then it would appear that there is present in his series of urines something which directly evokes expansion of melanophores.

Jores and his co-workers [1933–34], in a series of papers on urines, made observations which led them to conclude that, when using isolated frog skin, a positive reaction may be unspecific. There is one observation of Cunningham-Dax which suggested that a reinvestigation of this problem might be fruitful if reliable assay methods were available. He claimed that after treatment with charcoal the urines of his pathological cases no longer evoked melanophore expansion. Consequently

we have examined various urines using hypophysectomised *Xenopus* as a test animal.

A number of fresh urines from apparently normal men and women were neutralised and injected immediately into both normal and hypophysectomised *Xenopus.* Most of the samples evoked a slight melanophore expansion in normal animals, but only a few had any significant effect on hypophysectomised toads. One sample in the latter group was very potent, and its injection resulted in the test animals remaining dark for 24 hours. We treated large quantities of this urine with charcoal and found that after filtration the urine was still as potent as previously. We were not able to extract any active principle from the charcoal even though the phenol elution was prolonged for 24 hours at 50° C. As a test of method for small quantities of hormone, 50 gamma of our standard powder was added to 200 c.c. of the neutralised urine. Over 60 p.c. of this 50 gamma was extracted when phenol elution was prolonged for 24 hours at 50° C. While these tests are not conclusive they indicate that B hormone is not excreted in the urine, and that the melanophore excitant in some urines is another substance which affects the melanophores.

Further attempts to detect B in urine were made on mice and toads (*Bufo*).

Immature female mice with the vagina not yet open were placed on a wire gauze over a funnel as described by Burn [1931] for anti-diuretic assay. Two B preparations were used. The first was a highly potent 5 p.c. ox posterior-lobe extract prepared by the method of Hogben and Gordon. 2 c.c. of this were injected into each mouse. The yield of urine obtained, compared with that from the same animals injected with an equivalent bulk of saline, showed that, as measured by the bulk of urine produced, the extract had no anti-diuretic activity. After neutralisation and concentration the urine was injected into hypophysectomised *Xenopus.* It evoked no response in the melanophores.

In another experiment 12 mg. of the carbon-extracted material described in this paper were dissolved in 12 c.c. distilled water and 2 c.c. injected into each of 6 mice. No anti-diuretic activity was observed and the total yield of urine after 4 hours was concentrated and injected into *Xenopus.* The response was again negative.

Male *Bufo* with empty bladders were injected intraperitoneally with 2 c.c. distilled water containing 1000 gamma of B powder. Male *Bufo* were selected so that the results should not be vitiated by fluid pouring directly down the oviducts. The toads were placed in a dry glass vessel to collect the urine. It is our experience that *Bufo* so treated do not urinate for many hours unless stimulated. Urine can be pressed out, but it is more satisfactory to hold the toads over a vessel and apply a weak stimulus from an induction coil to the body-wall. This results

in an immediate and complete flow of urine. Urine collected 3 hours after injection evoked no response when injected into *Xenopus*. The amount of substance injected into each of the group of 6 mice was sufficient to fully darken 2000 *Xenopus*, and that injected into each *Bufo* was sufficient to darken 1000.

These results suggest strongly that B is not excreted in the urine. The following observations on *Rana*, *Xenopus* and *Anguilla* [Waring and Landgrebe, 1941; Waring and Clark, unpublished] lend some support to the view that B is destroyed by some substance normally present in the tissues. These animals were kept in black tanks with overhead illumination so that the melanophores were fully expanded. They were completely pithed and the heart canulated. Physiological saline was perfused and the melanophores contracted. B-containing extract was added to the perfusate and the melanophores expanded. When straight saline was again perfused through the preparation the melanophores did not contract. This implies one of two things: either the perfusing fluid does not permit the passage of the hormone *back* through the capillary walls, or the first perfusion removed from the tissues some substance which normally destroys B. If B hormone is not excreted in the urine, it differs in this respect from other pituitary autacoids already investigated. The urine of menopausal or ovariectomised women contains abundant gonadotropic substance which is almost certainly of anterior lobe pituitary origin. Jones and Schlapp [1936] found that within 20 minutes of injecting vasopressin and oxytocin into a decapitate cat 85 p.c. of the hormones had disappeared from the blood. Some of the loss could be attributed to destruction by various body tissues, but 30 p.c. of the vasopressin could be recovered from the urine. Larsen [1939] injected posterior pituitary extract into cats. About 27 p.c. of the oxytocic principle was recovered from the urine. He found that tissue polypeptidases inactivate the oxytocic principle. Heller [1940] has shown that anti-diuretic properties are not due to pressor autacoid as is generally believed. In a previous paper [1939] he showed that blood or liver contains an enzyme which destroys the anti-diuretic hormone, but that the latter could be detected in the urine up to 30 minutes after injection into the body.

VIII. The Physiological Rôle of B Hormone.

It is a significant fact that animals without active chromatophores have a rich store of B hormone in the pituitary and that this substance can be detected in the circulation.

Investigations of the pharmacodynamic influence or physiological rôle other than that of the co-ordination of chromatic response up to the present time may be considered under two main heads.

1. *Comparison of the Effects evoked by whole Posterior-lobe Extracts (containing B) and by relatively pure Extracts of B.*

(*a*) Injections of whole posterior extracts evoke a variety of responses. Few of these have a proved physiological significance [Van Dyke, 1940; Fisher *et al.*, 1938]. When the list was somewhat smaller and consisted of pressor, renal, oxytocic, and melanophore effects, Abel [summarised 1930] was of the opinion that these were all manifestations of the activity of one parent molecule. He considered that fractions containing a preponderance of one activity over the others were produced by the formation of cleavage products. Most authors now accept the simpler view that the posterior pituitary manufactures several different autacoids. Stehle [1938] has recently reviewed the question in detail. Two fractions are in common use—vasopressin (Parke Davis, Pitressin) and oxytocin (Parke Davis, Pitocin). Well-authenticated effects evoked are as follows [for original reference see Geiling, 1935; Stehle, 1938; and Van Dyke, 1940]: Pitressin affects blood-pressure, renal urine flow, and water balance [see Boyd and Hicks, 1940; Dow and Zuckermann, 1939], peristalsis of the lower intestine, oxygen consumption, movements of the uterus in some species, expansion of melanophores. Oxytocin evokes constriction of uterine muscle, has a depressant action on the blood-pressure of birds, a relaxing effect on the coronary arteries, and some melanophore activity.

Both vasopressin and oxytocin may produce a hyperglycæmia. General extracts antagonise the hypoglycæmic action of insulin and the hyperglycæmic action of adrenalin. They have a marked effect on the evacuation of milk from the mammary glands [Foley, 1940]. There is some evidence that extracts affect the metabolism of lipoids and proteins. Various effects have been reported as a result of posterior-lobe injection which, in the absence of evidence to the contrary, may be taken to result directly from increased blood-pressure or vaso-constriction [Van Dyke, 1940].

Pitressin contains abundant melanophore hormone and pitocin little. So if the melanophore substance is responsible for any of the recognised effects of whole extracts, these are to be sought in the first place among effects evoked by the former. On the other hand, it is conceivable that B is antagonistic to pressor substance, in which case no clue to its rôle would be furnished by this approach. Stehle and Fraser [1935] have recently prepared a pressor fraction substantially free from B. Its other properties have not been reported on.

(*b*) The first systematic attempt to test whether any of these effects were due to intermediate lobe hormone were made by Zondek and Krohn [1933]. They stated that their product Intermedin does not affect the mobility of the uterus, blood-pressure, bladder or intestine, diuresis, blood sugar, glycogen or fat content of liver, basal metabolism.

Gaddum [1928] had previously shown that caustic-treated (*i.e.* pressor-free) posterior-lobe extracts had no effect on the intestine. The relation of B to blood sugar, renal flow, and metabolic rate has been reinvestigated by later workers.

Jores [1935] claimed that injections of melanophore preparation produced a fall in temperature and a rise in blood sugar. It seems likely, however, that his extract was contaminated, for, quite apart from Zondek's observations, Geiling and Eddy [1928] had previously shown that the hyperglycæmia produced by pituitary injection did not occur if the extract was treated with alkali, and we know that "B" is resistant to alkali. Sulzberger [1933, 1935], Dietel [1934], and Böttger [1936] have claimed an anti-diuretic effect after injection of melanophore-hormone preparations into unanæsthetised animals and man, but later work [Sulzberger, 1936; Fraser, 1937; and Böttger, 1937] was taken to imply that the effects observed were due to contamination of their extracts with pressor substance which until recently was considered to be responsible for the anti-diuretic effect of extracts injected into unanæsthetised animals. Heller [1939, 1940] has now shown that the anti-diuretic effect is due to a substance separable from pressor autacoid by its heat resistance. Apparently both substances are destroyed by caustic soda. Heller attributes the diuresis which accompanies the injection of posterior extracts into anæsthetised animals to the pressor autacoid. Although this hypothesis has a rational basis, it has not been controlled by injection of B alone. The fact that Sulzberger used Intermedin and obtained a result different from Zondek and Krohn in regard to diuresis might be taken to imply that other statements of these authors would benefit by independent confirmation.

(*c*) Posterior-lobe extracts evoke a very definite metabolic effect even in the absence of the thyroid. This fact has recently been the focus of extensive investigations by Collip *et al.* [1937–39], Teague [1939], and Feinstein and Gordon [1940]. O'Donovan and Collip [1938] observed an immediate metabolic stimulation in mammals as a result of injecting extracts rich in B. The active principle could be distinguished from the thyrotropic, adrenotropic, and growth hormones of the A.L.P., and from the pressor and oxytocic principles. It was similar to B in its resistance to alkalies and in its anatomical distribution. Teague [1939] observed that preparations rich in melanophore hormone obtained from various sources and prepared by different methods vary considerably in their effect on oxygen consumption in rats. More important still, he found that when one commercial preparation was subjected to HCl or tryptic digestion, the product produced even more consistent metabolic stimulation than the original extract. Teague also observed occasional stimulation of metabolic rate by injection of muscle, liver, and kidney extract. Denstedt and Collip [1940] have now agreed that pituitary fractions can be obtained in which the melano-

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In addition to the investigations cited there are a few miscellaneous reports which do not fall into either of the above categories. Holmquist [1934] and Jores and Beck [1934] reported that repeated injection of B-containing extracts resulted in hypertrophy of the adrenal cortex. Quite apart from the fact that their extracts may have been contaminated with A.L.P., it is known from the work of Preston [1928], Deanesly [1930], and Hoerr [1931] that a wide variety of substances evoke cortical hypertrophy. Dietel [1934] has described capillary dilatation following treatment with melanophore hormone.

Other Possible Correlations with B Hormone Activity.

In spite of the extent and variety of the investigations referred to, exploration of the physiological rôle of B is still far from complete. The lines of investigation summarised above are by no means exhausted. In addition, there are suggestive data of another kind which have received little or no attention; for example—

(*a*) Judging by the method of preparation, most anterior pituitary extracts are contaminated to some extent with B. The latter may possibly play a significant part by acting synergistically with, or antagonistically to, the dominant autacoid of the extract.

(*b*) Waring [1936 *b*] described a seasonal variation in the B content of the pituitary of *Rana*. Masselin [1939] has since confirmed this for *Bufo*. In *Rana* there is a sharp fall in potency of extracts prepared at about the middle of March. This correlation between a change of pituitary B content and the peak of sexual activity suggests that they may not be unrelated.

(*c*) MacCallum *et al.* [1935], in a detailed post-mortem description of a 25-year-old female who exhibited symptoms of a typical Cushing syndrome, concluded that the pituitary tumour present was derived from *pars intermedia* cells. They point out that, if the symptoms result from the enlargement of the *pars intermedia*, they may be due either to compression of the anterior lobe or to enlargement of the intermedia as such. They consider the former to be unlikely, because other cases in their experience exhibited greater compression of the anterior lobe without evincing any of the symptoms noted.

(*d*) A prospect of great potential interest is opened up by the observations of Sumner and Doudoroff [1938] on the mortality of fish maintained on different backgrounds. They obtained clear-cut evidence that fish kept for long periods on a black background with overhead illumination showed a higher incidence of a fatal bacilliary infection than similar

phore hormone and metabolic principle do not parallel one another. Feinstein and Gordon [1940] concluded "that though significantly increased metabolism is occasionally seen after the injection of similarly prepared extracts of liver, the data appear sufficient to warrant the conclusion that the pituitary does contain a metabolic principle which cannot be obtained from similarly prepared extracts of liver." These authors' give no details of the pituitary extracts used. While the existence or otherwise of a specific metabolic stimulant in the pituitary is of undoubted importance, Teague's [1939] observation that the metabolic stimulation is still present after tryptic digestion furnishes unequivocal evidence that the substance is not B.

2. *Potential Effects due to the fact that B controls Melanophore Activity.*

(*a*) Jores [1933] claimed that there was a migration of pigment after the instillation of B-containing extracts into the human eye, and the vision of the treated eye was thought to show more rapid dark adaptation than the control [Jores and Hotop, 1934]. Their findings were not confirmed by Buschke [1934] using material supplied to him by Jores. Jores also claimed that there is an increased B level in the blood of rabbits in the dark. While this may be taken as supportive evidence for the first claim, it is so different from the condition holding in Amphibia that it requires confirmation. Jores and Cæsar [1935] also claimed that pigment in the retina of the frog's eye assumes the dark position at a more rapid rate if the eye is treated with a solution of B. But hypophysectomy does not alter the movement of retinal pigment in response to light or darkness in either frogs [Jores and Cæsar, Matuo, 1935] or toads [Okamato, 1937]. There is clearly too much actual and implied contradiction in these observations to draw any conclusion. Our general objection to all Jores's work is that we are not satisfied that his assay methods are adequate or his extracts pure enough.

(*b*) Recently [Fostvedt, 1939–40] there has appeared suggestive evidence for the participation of the melanophore hormone in melanin formation. Indirect support for this is furnished by the fact that when chromatically active animals are maintained for long periods on an illuminated black background (*i.e.* when exposed to maximum concentration of effective B) there is an absolute increase in melanin, and on a white background an absolute loss [summary, Sumner, 1940]. Teague [1939] has pointed out that oxygen is utilised in the tyrosine-tyrosinase oxidase system in the production of melanin *in vitro*. Fostvedt showed this system is accelerated by melanophore hormone. If this occurs *in vivo* it may be the basis for the observed increase of oxygen consumption after injection of pituitary extract.

caustic, is adsorbed by charcoal, and is now eluted, together with melanophore-dispersing hormone, by phenol. This substance may be oxytocin.

8. Since the alterations brought about by caustic treatment (paragraph 6 above) in the melanophoric properties of an extract depend on the effect of the alkali on some pituitary constituent other than B, quantitative estimations of the B content of pituitary substance or of body fluids (*e.g.* blood) cannot furnish reliable data if caustic soda is used in the preparation of the extracts injected into the test animal.

9. Evidence is presented to substantiate the view that melanophore-dispersing hormone is not passed by the kidney but is destroyed in the tissues. We were unable to detect the presence of B hormone in the urine of normal human beings.

10. Recent findings on the physiological rôle or pharmacodynamic effect of B hormone, other than the co-ordination of chromatic response, are briefly reviewed.

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fish on a white background. Since in their experiments no extraneous substances, *e.g.* paint, were in contact with the water, adequate oxygenation was provided, and the fish in both containers consumed approximately the same quantity of food, it appears probable that a sustained high level of B in the circulation may bring about a condition specially favourable to the multiplication of at least one kind of bacillus.

IX. Summary.

1. The optimum source for the manufacture of melanophore hormone (B hormone) is the posterior lobe of the pituitary, provided that the pressor and oxytocic autacoids can be removed without recourse to caustic soda treatment.

2. Previous methods of assaying melanophore activity are criticised, and the advantages of hypophysectomised *Xenopus lævis* as a test animal are emphasised.

3. A provisional unit of melanophore activity is proposed.

4. A new and rapid method is described for the preparation of B extracts which are substantially free from pressor substance (less than 1 unit of pressor activity in 4000 melanophore units) and oxytocin (less than 1 unit in 40,000 melanophore units). The final product in powder form keeps indefinitely and may be standardised on a weight basis with fair accuracy.

5. The preparation of B-containing pituitary extracts is examined in detail. The oxytocic, pressor, and melanophore principles from posterior-lobe extract are adsorbed by active charcoal. In 1 hour 25 p.c. of the melanophore activity can be eluted from the charcoal with pure water-free liquid phenol. In this time little or no vasopressin and oxytocin are eluted.

6. The rôle of caustic soda, hitherto widely used to remove pressor and oxytocic properties from B-containing extracts, is explored. Caustic soda treatment of general extracts modifies the melanophoric properties in at least two ways: (*a*) the melanophore-expanding potency is increased; (*b*) there is an increased duration of the response when sub-maximal doses are injected.

7. Differences in the response curves of hypophysectomised *Xenopus* to caustic treated and untreated whole pituitary extracts are not primarily due to the destruction of the pressor activity. In crude posterior-lobe extracts there is a substance other than pressor autacoid or B-precursor which, after treatment with caustic, modifies the melanophore response evoked by B hormone in the two ways noted. This substance is adsorbed on to charcoal from untreated extracts but is not eluted in 1 hour by phenol. After treatment of the general extract with caustic soda this substance, presumably modified by

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CHANGES IN THE SECRETORY ACTIVITY OF THE GASTRIC GLANDS RESULTING FROM THE APPLICATION OF ACETIC ACID SOLUTIONS TO THE GASTRIC MUCOSA.

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Several theories have been advanced to explain the mechanism of the regulation of the acidity of the gastric juice. They range from the purely physiological, such as that the concentration of acid in the juice depends on the strength of the stimulation applied to the glands, to the purely physico-chemical, such as that the variations in the gastric acidity are due to a simple exchange of ions between the gastric contents and the blood. Nevertheless the problem of the regulation of the gastric acidity is still far from solution. An interesting study by the late Professor V. V. Savich and co-workers stimulated us to return to the investigation of this problem.

Working on dogs with a Pavlov or a Brestkin-Savich (innervated) gastric pouch, Gorbunova, Lebedinskaïa and Savich [1933] demonstrated that, after the introduction into the main stomach of 300 c.c. of 1 per cent. acetic acid, there was a marked change in the secretory response of the pouch to ingestion of milk or of a 5 per cent. solution of Liebig's Extract, or to a rectal injection of 6 per cent. alcohol. On the day after the administration of acetic acid, when the pouch secretion was tested in any of the above-mentioned ways, it was found that the secretion was diminished in volume and that it contained much mucus and had a lower acidity. On the third day the mucus content of the secretion was reduced, but the acidity of the juice still remained below normal. These experiments showed that the acetic acid acted not only on the mucous membrane with which it came in direct contact but also on the mucosa of an isolated pouch *par distance*: that is, the important fact established by Gorbunova, Lebedinskaïa and Savich was that as a result of the introduction of acetic acid into the main stomach the juice secreted by the pouch was diminished in volume and altered in composition. That was what interested us most in this work. For a number of years it has been held in our laboratory that the variations so often occurring in the composition of

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solution lost was replaced and the experiment continued. In one of the dogs with œsophagotomy and a gastric fistula the effect of introduction into the stomach of 2·2 per cent. lactic acid was also tested. Control experiments were performed before and for several days after administration of the acid solutions.

The total and free acidities of the gastric juice were determined by titration with ·02 N solution of NaOH, phenolphthalein and Töpfer's reagent being used as indicators. The total chloride was estimated by Wilson and Ball's [1928] modification of Van Slyke's method and the values were expressed in milli-equivalents per litre. For the determination of the peptic power Nirenstein and Schiff's modification of Mett's method [Hawk and Bergeim, 1937] was employed. To determine the amount of mucus in the gastric juice, samples were centrifuged for 5 minutes at constant speed and the volume of the sediment measured.

Results.

The results of experiments on two of the dogs with œsophagotomy and a gastric fistula (Dogs K and T) are shown in Tables I. and II.

Table I.

Dog K (dog with œsophagotomy and a gastric fistula).—*H.* = 0·6 mg. of histamine phosphate injected subcutaneously. *Sh.F.* = 5 minutes' sham-feeding with meat. In the experiments in which acetic acid solution was previously introduced into the stomach, histamine was injected or sham-feeding performed 20 minutes after the stomach had been emptied of the acetic acid. In the experiments of Dec. 18 and Jan. 15 the dog vomited part of the acetic acid solution, which was collected and re-introduced into the stomach.

Date of expt.	Stimulus.	Volume of gastric secretion, c.c.	Mucus content, c.c.	Free acidity, m.eq./l.	Total acidity, m.eq./l.	Total Cl concent., m.eq./l.	Peptic power, Mett units.	Remarks.
Dec. 11	*H.*	66·5	0·6	131	143	164	33	
„ 13	*H.*	80·7	1·1	133	145	169	33	
„ 18	*H.*	*58·2*	*13·4*	*45*	*69*	*137*	*15*	Before histamine administration, 250 c.c. 1 per cent. *acetic acid* introduced into stomach for 2 hrs. 25 min.
„ 20	*H.*	60·9	1·3	133	142	167	20	
Jan. 8	*Sh.F.*	65·8	4·2	124	142	166	200	
„ 10	*Sh.F.*	39·4	2·8	120	137	166	208	
„ 15	*Sh.F.*	*91·2*	*11·0*	*54*	*76*	*137*	*50*	Before sham-feeding, 200 c.c. 1 per cent. *acetic acid* introduced into stomach for 2 hrs. 25 min.
„ 17	*Sh.F.*	55·1	3·5	122	137	167	200	
„ 23	*Sh.F.*	*41·3*	*11·9*	*74*	*92*	*150*	*96*	Before sham-feeding, 100 c.c. 1 per cent. *acetic acid* introduced into stomach for 1 hr.

Dog K (Table I.).—In all the experiments where variable amounts of 1 per cent. acetic acid solution were previously introduced into the

the gastric juice are due chiefly to changes in the secretory activity of the different cellular groups which form part of the gastric glands [*cf.* Babkin, 1931, 1934, 1938 *a* and *b*]. A good means of testing this theory was offered by acetic acid, a stimulant which is capable of altering the functional activity of the gastric mucosa. Such an investigation was the more desirable since Gorbunova, Lebedinskaïa and Savich did not determine systematically the total chloride, neutral chloride and pepsin of the gastric juice, all three of which are extremely important components of the secretion. For this reason, it may be remarked, their theory that the secretion of water and the secretion of acid by the gastric glands are two distinct functions would require further investigation.

Methods.

The experiments were all of the chronic type and were performed on five dogs, K, T, B, C, and H. Dogs K, T, and B were œsophagotomised dogs provided with a metal gastric fistula. Dogs K and B were otherwise perfectly normal animals. In Dog T (for special reasons unconnected with this work) all the gastric arteries were tied and cut on Aug. 3. [This procedure has been carried out on several dogs in our laboratory by Drs. J. C. Armour and D. R. Webster (results not yet published), who showed that it does not interfere with the secretory function of the gastric glands when they are stimulated by sham-feeding or histamine. Even a week or two after the ligature of the arteries the secretory response to either stimulus was almost normal.] The experiments with acetic acid on Dog T were started on Sept. 24, 52 days after the operation.

The secretion of gastric juice was stimulated in the dogs with œsophagotomy and a gastric fistula by sham-feeding with meat for 5 to 15 minutes or by subcutaneous injection of histamine, and the direct effect of previous administration of acetic acid on the reflex phase of gastric secretion was studied. In Dog C, which was equipped with a Pavlov pouch and a metal fistula in the main stomach, acetic acid solution was introduced into the main stomach and the secretory response of the pouch to a test-meal of meat or to rectal injection of 200 c.c. of 5 per cent. ethyl alcohol was studied. Dog H was provided with a Bickel pouch (*i.e.* one deprived of all extrinsic innervation) and a metal fistula in the main stomach. In this animal the effect of the introduction of acetic acid into the main stomach was reflected on the secretory function of the denervated pouch during the second (or pyloric) and third (or intestinal) phases only.

In all these animals from 100 to 300 c.c. (according to body-weight) of a 1 per cent. solution of acetic acid were introduced through the metal fistula into the stomach and kept there for from two to three hours, after which the stomach was emptied and the sham-feeding or other gastric stimulus applied. Usually about one-fifth of the acetic acid solution was recovered from the stomach; the rest passed into the duodenum and was presumably absorbed. In a few experiments the dogs vomited a small amount of the acetic acid solution. In such cases the experiment was discontinued, or the quantity of

obtained uniform results. Undoubtedly acetic acid, on being applied locally to the gastric mucosa, caused a great secretion of mucus, whereas in control experiments, if there was no secretion of gastric juice before histamine administration or sham-feeding, a scanty amount of mucus mixed with acid was discharged from the gastric fistula. The volume of this secretion of mucus was 0·2 to 0·6 c.c. per 15 minutes. After the administration of acetic acid the volume of mucoid secretion (in the interval between the emptying of the stomach of acid solution and

TABLE II.

Dog T (dog with œsophagotomy and a gastric fistula).—*H.* = subcutaneous injection of histamine phosphate. *Sh.F.* = 5 minutes' sham-feeding with meat. In the experiments in which acetic acid solution was previously introduced into the stomach, histamine was injected or sham-feeding performed 15 minutes after the stomach had been emptied of the acetic acid. In the experiments of Oct. 2, Oct. 10, and Nov. 1 the dog vomited about 50 c.c. of the acetic acid solution, which was re-introduced into the stomach.

Date of expt.	Stimulus.	Volume of gastric secretion, c.c.	Mucus content, c.c.	Free acidity, m.eq./l.	Total acidity, m.eq./l.	Total Cl concent., m.eq./l.	Peptic power, *Mett units.*	Remarks.
Sept. 24	*Sh.F.*	88·2	3·2	125	136	158	138	
Oct. 2	*Sh.F.*	*47·5*	*4·1*	*59*	*75*	*146*	*44*	Before sham-feeding, 250 c.c. 1 per cent. *acetic acid* introduced into stomach for 2 hrs. 25 mins.
,, 4	*Sh.F.*	62·2	2·8	120	135	161	187	
,, 8	*Sh.F.*	53·1	1·8	115	129	160	188	
,, 10	*Sh.F.*	*63·6*	*3·1*	*80*	*97*	*148*	*117*	Before sham-feeding, 250 c.c. 1 per cent. *acetic acid* introduced into stomach for 2 hrs. 25 mins.
,, 11	*Sh.F.*	88·9	1·2	112	127	162	112	
,, 16	*H.*	84·7	1·3	132	143	162	78	1 mg. histamine phosphate injected.
,, 18	*H.*	*34·7*	*4·3*	*72*	*89*	*153*	*35*	Before injection of 1 mg. histamine phosphate, 250 c.c. 1 per cent. *acetic acid* introduced into stomach for 2 hrs. 25 mins.
,, 30	*H.*	119·4	1·3	137	147	168	31	0·75 mg. histamine phosphate injected.
Nov. 1	*H.*	*45·3*	*6·8*	*71*	*90*	*151*	*70*	Before injection of 0·75 mg. histamine, 250 c.c. 1 per cent. *acetic acid* introduced into stomach for 2 hrs. 25 mins.
,, 13	*H.*	39·7	0·9	124	138	171	71	0·5 mg. histamine phosphate injected.
,, 15	*H.*	*69·4*	*6·1*	*90*	*108*	*150*	*27*	Before injection of 0·75 mg. histamine phosphate, 250 c.c. 1 per cent. *acetic acid* introduced into stomach for 2 hrs. 25 mins.
,, 20	*H.*	37·1	1·3	123	142	166	87	0·6 mg. histamine phosphate injected.

stomach and allowed to remain there for different periods of time, the subsequent response of the gastric glands to histamine administration or to sham-feeding with meat was uniformly altered. The gastric juice now differed from the normal "histamine" or "sham-feeding" juice in the following respects:—

(1) There was a *great increase in visible mucus*. (*N.B.*—The mucus content of the normal sham-feeding juice is usually greater than that of histamine juice.)

(2) The free and total acidities were much reduced.

(3) The total chloride concentration was reduced.

(4) The peptic power of both the sham-feeding and the histamine juice was diminished.

The volume of the secretion provoked by sham-feeding or by histamine was diminished after the application of acetic acid in most of the experiments, as we shall see later. However, in some cases, as in the experiment of Jan. 15 (Table I.), it was increased. Since this was an experiment involving sham-feeding, there was one factor—the appetite of the animal—which was difficult, if not impossible, to control. Nevertheless, in spite of the greater volume of secretion in this experiment, the free and total acidities, total chloride concentration, and concentration and total output of pepsin were lower than in the control experiments (*e.g.* experiments of Jan. 10 and 17), where the volume of secretion was only about half as large. A very instructive comparison may be drawn between this and the histamine experiments of Dec. 18 and 20, where the volumes of secretion in the control and the test experiments were practically equal (viz. 60·9 c.c and 58·2 c.c.).

Dog T (Table II.).—The experiments on this animal fully confirm the results obtained on the previous dog (K). Various doses of histamine were tried in order to produce a volume of gastric secretion equal to that of the control experiment, but the volume of the secretion in all the histamine experiments after the application of acetic acid was less than in the corresponding control experiments. Although in one of the sham-feeding experiments (Oct. 2) the acetic acid markedly diminished the volume of secretion, in another (Oct. 10) the inhibition was doubtful. This cannot be ascribed to the occasional vomiting of a small amount of acetic acid solution, because vomiting occurred in other experiments (Oct. 2 and Nov. 1) and yet there was no increase in the volume of secretion. In the same experiment (Oct. 10) there was a less marked reduction of the peptic power of the juice obtained after the preliminary introduction of acetic acid into the stomach than in the experiment of Oct. 2. We did not try to analyse this fact because our interest was centred on changes in the acidities and the concentration of chloride in the gastric juice and in the secretion of mucus by the gastric mucosa under the influence of acetic acid. In that respect in all eight of the acetic acid experiments on the two dogs, K and T, we

ments on Dog K, in which the gastric secretion was stimulated by the same dose of histamine injected subcutaneously with and without preliminary treatment with acetic acid. In all other experiments the same relations were observed, the difference between the volume of mucus secreted in control experiments and that secreted in experiments in which acetic acid was previously introduced into the stomach being

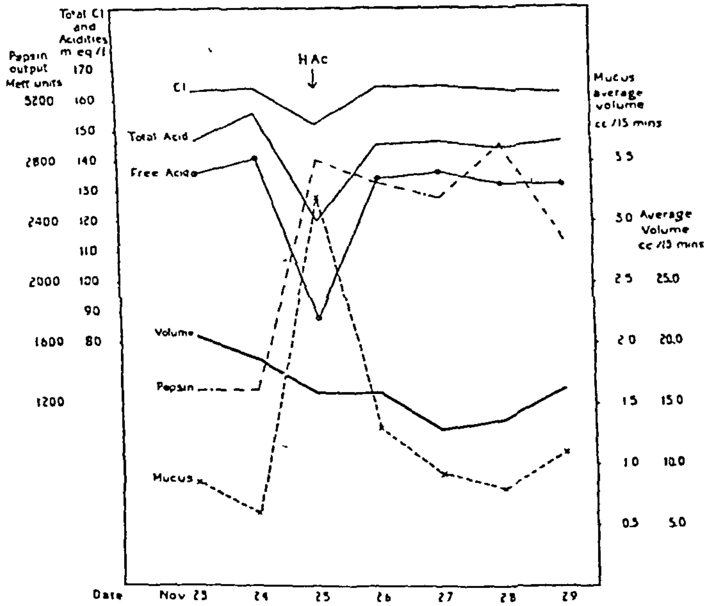

FIG. 1.—The effect of previous introduction of acetic acid solution into the stomach on the gastric secretory response to sham-feeding with meat in a dog with œsophagotomy and a gastric fistula.

less marked when the stimulus was sham-feeding than when it was histamine.

Dog B.—Prolonged experiments were performed on Dog B, also an œsophagotomised dog with a gastric fistula. The after-effects of a single introduction into the stomach of a 1 per cent. solution of acetic acid or of a 2·2 per cent. solution of lactic acid were studied in the case of each acid for several days. Fig. 1 illustrates a typical experiment which lasted 7 days. The gastric secretion obtained by 15 minutes' sham-feeding with meat was collected every day. Control observations had been made on the first two days and then on the third day 300 c.c. of 1 per cent. acetic acid solution were introduced into the empty stomach. After 2 hours and 25 minutes the gastric contents (47 c.c.) were removed through the opened fistula. Half an hour later sham-feeding with meat was performed in the usual manner. The effect of the acetic acid was striking. The volume of secretion diminished on the day

the beginning of sham-feeding or injection of histamine) was many times greater than that of the control for the same length of time. The following are the average figures for this mucoid secretion in all the experiments in which acetic acid was introduced in the two dogs:—

	Volume per 15 min., c.c.	Free acidity, m.eq./l.	Total acidity, m.eq./l.	Total Cl. concent., m.eq./l.	Peptic power, Mett units.
Dog K . .	5·9	23·7	56·9	125	38
Dog T . .	2·9	14·7	29·5	122	83

It will be noted that in Dog T the acidities and the concentration of the total chloride were lower and the peptic power somewhat higher than in Dog K.

During the course of the secretion induced by sham-feeding or histamine, the secretion of mucus, large at the beginning, gradually diminished (the first samples of gastric juice always contain more mucus). Nevertheless the total and free acidities and the total chloride even in the last samples of gastric juice obtained by sham-feeding or histamine after administration of acetic acid were much lower than in the control experiments. *Thus the lowering of the acidity and of the total chloride content did not depend only on the admixture of mucus with the gastric juice.* Some other factors presumably also played an important part in this phenomenon.

The reaction of the gastric glands to the irritating effect of acetic acid is illustrated in Table III., which shows the results of two experi-

TABLE III.

Dog K: Expt. Dec. 11.—Gastric secretion in response to subcutaneous injection of 0·6 mg. histamine phosphate.

Dog K: Expt. Dec. 18.—Gastric secretion in response to subcutaneous injection of 0·6 mg. histamine phosphate after preliminary introduction of acetic acid into stomach.

Volume, per 15 min., c.c.	Mucus content, per 15 min., c.c.	Free acidity, m.eq./l.	Total acidity, m.eq./l.	Total Cl concent., m.eq./l.	Peptic power, Mett units.	Volume, per 15 min., c.c.	Mucus content, per 15 min., c.c.	Free acidity, m.eq./l.	Total acidity, m.eq./l.	Total Cl concent., m.eq./l.	Peptic power, Mett units.
10·0	0·42	107	122	161	125	14·0	5·50	30	58	130	19
23·3	0·06	134	146	168	16	27·7	7·30	42	66	136	16
13·7	0·06	136	147	165	10	10·5	0·45	66	91	142	10
12·5	0·08	138	150	163	23	4·3	0·13	64	89	145	10
7·0	0·03	136	146	158	19	1·7	0·05	52	76	142	13
0·7	0·00	89	119	..	..	..	..	..	..	..	..
67·2	0·65	131	143	164	33	58·2	13·43	45	69	137	20

acetic acid had left the stomach and the gastric secretion of the pouch had ceased. Acetic acid itself elicited from the Pavlov pouch a secretion of 5 to 8 c.c. of gastric juice, having a somewhat lower concentration of total chloride (152 to 158 m.eq. per litre), and usually a lower free acidity (86 to 94 m.eq. per litre) and total acidity (105 to 110 m.eq. per litre) than the secretion in control experiments with meat. The concentration, and often the volume of mucus in the pouch secretion, was increased, while the peptic power of the juice was moderate. In the

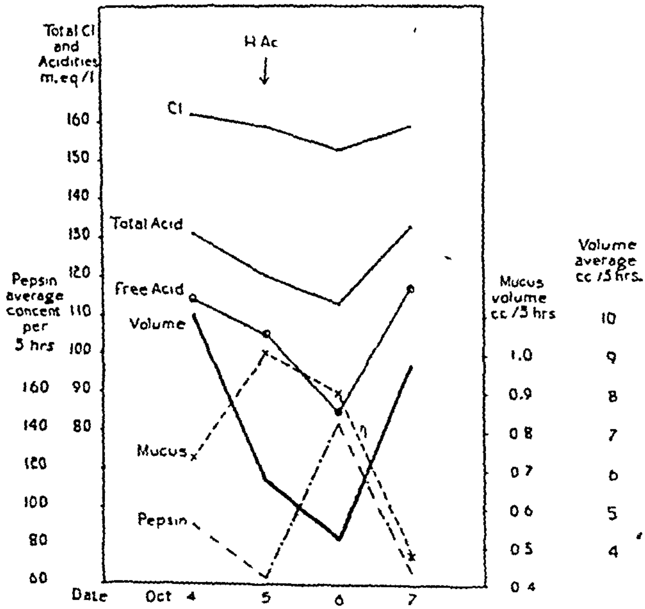

Fig. 2.—The effect of previous introduction of acetic acid solution into the main stomach on the secretory response of a Pavlov pouch to a test-meal of meat in a dog.

fifth series, after the introduction of acetic acid solution into the stomach, 200 c.c. of 5 per cent. alcohol were injected into the rectum and the gastric secretion was collected from the pouch.

The facts observed by Gorbunova, Lebedinskaïa and Savich were confirmed, viz. that after the introduction of acetic acid into the main stomach the Pavlov pouch responded to a test-meal of meat or to a rectal injection of alcohol with a smaller gastric secretion, containing a greater amount of mucus and possessing a lower acidity and a lower total chloride concentration. No marked changes were noted in the peptic activity of the juice.

Fig. 2 represents one of a series of typical experiments. The greatest depression of the curves for the total chloride concentration, for both free and total acidity and for volume of the secretion, occurred on Oct. 6, the day after administration of acetic acid. This coincided

that the acetic acid was administered (Nov. 25) and continued reduced until the fourth day, after which it began to rise. The volume of visible mucus was five times greater after acetic acid on the day of its administration. In some of the samples of juice collected on that day it formed half of the fluid. The volume of mucus fell sharply the next day and the following day returned to normal. The total chloride concentration and the free and total acidities were markedly reduced on the day that acid was administered; they all returned practically to normal within the next two days (Nov. 26 and 27). A peculiar effect of acetic acid administration on the peptic power of the juice was observed in this dog. Both the concentration and the output of pepsin rose sharply, remained high for four days, and then began to fall. The results of this experiment leave no doubt that the changes in the composition of the gastric juice were due to the increased activity of the mucus-producing and perhaps of some other glandular cells caused by the acetic acid, and not to the diminished volume of the gastric secretion.

The introduction into the main stomach of 300 c.c. of 2·2 per cent. lactic acid in the same dog had a somewhat different effect on the gastric secretion elicited by a subsequent sham-feeding of meat. The volume of secretion was not diminished, but on the first day tended to rise slightly. The volume of mucus secreted was tripled or quadrupled on the day of introduction of the lactic acid, but diminished thereafter. Correspondingly the concentration of total chloride and the total and free acidities fell slightly on the first day, but next day had already returned to normal or almost normal. No definite effect was produced by the lactic acid on the concentration and output of pepsin in the sham-feeding secretion.

Dog C (*Pavlov-pouch Dog*).—Gorbunova, Lebedinskaïa and Savich (*l.c.*) observed that the introduction of 300 c.c. of 1 per cent. acetic acid into the main stomach of dogs with an innervated gastric pouch elicited from the pouch a scanty secretion containing a great amount of mucus. On the second day, 100 c.c. of 6 per cent. ethyl alcohol were administered per rectum, which produced from the pouch a secretion of gastric juice having a low acidity and a high mucus content. On the third day alcohol was again injected and produced from the pouch a secretion in which the mucus content was practically normal but the acidity still low.

We repeated these experiments of Gorbunova, Lebedinskaïa and Savich on a dog with a Pavlov pouch. Five series of experiments, each series lasting several days, were performed on this animal. In four series 300 gm. of minced beef, alone or mixed with 200 c.c. of water, were used as a test-meal. About 2 to $2\frac{1}{2}$ hours before the meat was given, 200 or 300 c.c. of 1 per cent. solution of acetic acid were introduced into the main stomach. By the end of this period most of the

acetic acid had left the stomach and the gastric secretion of the pouch had ceased. Acetic acid itself elicited from the Pavlov pouch a secretion of 5 to 8 c.c. of gastric juice, having a somewhat lower concentration of total chloride (152 to 158 m.eq. per litre), and usually a lower free acidity (86 to 94 m.eq. per litre) and total acidity (105 to 110 m.eq. per litre) than the secretion in control experiments with meat. The concentration, and often the volume of mucus in the pouch secretion, was increased, while the peptic power of the juice was moderate. In the

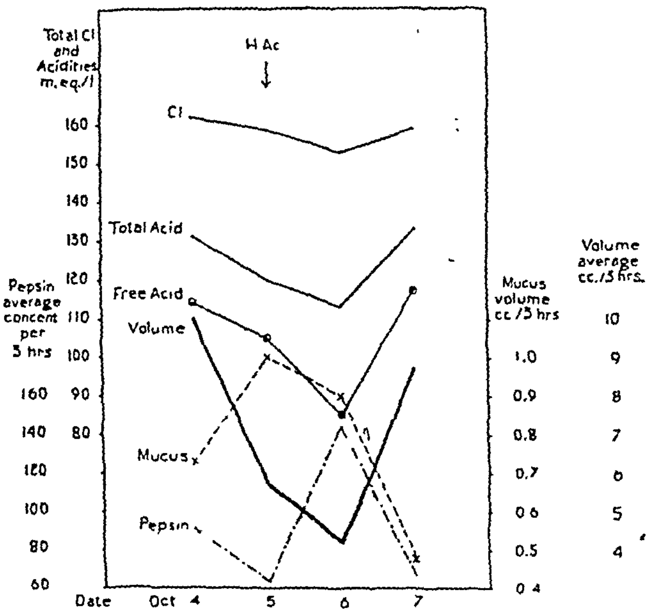

FIG. 2.—The effect of previous introduction of acetic acid solution into the main stomach on the secretory response of a Pavlov pouch to a test-meal of meat in a dog.

fifth series, after the introduction of acetic acid solution into the stomach, 200 c.c. of 5 per cent. alcohol were injected into the rectum and the gastric secretion was collected from the pouch.

The facts observed by Gorbunova, Lebedinskaïa and Savich were confirmed, viz. that after the introduction of acetic acid into the main stomach the Pavlov pouch responded to a test-meal of meat or to a rectal injection of alcohol with a smaller gastric secretion, containing a greater amount of mucus and possessing a lower acidity and a lower total chloride concentration. No marked changes were noted in the peptic activity of the juice.

Fig. 2 represents one of a series of typical experiments. The greatest depression of the curves for the total chloride concentration, for both free and total acidity and for volume of the secretion, occurred on Oct. 6, the day after administration of acetic acid. This coincided

with the highest concentration of mucus in the juice (Oct. 4, 7·4 per cent.; Oct. 5, 18·5 per cent.; Oct. 6, 20·0 per cent.; and Oct. 7, 5·5 per cent.), although the total volume of mucus on Oct. 6 was somewhat less than on the day that acetic acid was administered.

These effects of acetic acid were also quite marked during the late afternoon gastric secretion, provoked by a second feeding of the animal after the cessation of the secretion on meat. The second meal consisted of oatmeal *porridge (100 g. of oatmeal cooked in* 200 c.c. of milk and 100 c.c. of water, with the addition of 1 g. of sodium chloride) and the gastric juice from the pouch was collected over a period of 6 hours. The following data were obtained on the same days that the main experiment (shown in fig. 2) was performed:—

Date.	Volume, c.c.	Free acidity, m.eq./l.	Total acidity, m.eq./l.	Mucus content, per cent.	Remarks.
Oct. 4	35·0	121	137	8·1	
,, 5	*18·0*	*94*	*115*	*23·0*	Acetic acid solution introduced into main stomach in the morning.
,, 6	5·4	44	64	54·2	
,, 7	12·0	83	104	31·0	

It is evident from both series of experiments that the greatest depression of the pouch secretion and the proportionally greater content of mucus were observed not on the day of acetic acid administration but on the following day.

These experiments, in confirmation of the data of Gorbunova, Lebedinskaïa and Savich, show that, when a solution of acid is introduced into the main stomach, it acts not only on the gastric mucosa with which it comes in direct contact but also in some unknown way on the mucosa of the Pavlov pouch, which is completely separated from the main stomach. However, the effect of preliminary administration of acetic acid on the volume and the composition of the histamine or sham-feeding gastric secretion *par distance* in the Pavlov-pouch dog was not so immediately noticeable as had been its local effect in the œsophagotomy dogs, although in the former it seemed to last somewhat longer.

It might be objected, in regard to the experiments involving sham-feeding or ingestion of meat, that the animals perhaps lost their appetite after administration of the acetic acid solution and that this may have affected the volume and the composition of the gastric secretion. T supposition is invalidated by the experiments on the Pavlov-pouch in which gastric secretion was provoked by the rectal injectio

200 c.c. of 5 per cent. ethyl alcohol before and after introduction into the main stomach of 300 c.c. of 1 per cent. acetic acid (Table IV.).

TABLE IV.

Dog C (*Pavlov-pouch Dog*).—Illustrating the effect of previous introduction into the main stomach of 300 c.c. of 1 per cent. acetic acid solution on the pouch secretion elicited by rectal injection of 200 c.c. of 5 per cent. ethyl alcohol. In the experiment of Feb. 3 acetic acid was kept in the stomach for 3 hours; alcohol was injected 30 minutes after the stomach had been emptied of acid. With the exception of those for total volume of secretion and percentage of mucus, the figures represent the average per hour of secretion.

Date of expt.	Stimulus.	Total volume, c.c.	Average volume, c.c./hr.	Mucus content, per cent.	Free acidity, m.eq./l.	Total acidity, m.eq./l.	Cl concent., m.eq./l.	Peptic power, Mett units.
Feb. 2	Alcohol	9·5	3·2	2·1	109	132	154	74
Feb. 3	Acetic acid	8·2	2·7	2·4	104	122	157	80
	Alcohol	8·5	2·8	5·3	93	108	153	52
Feb. 4	Alcohol	10·3	3·4	3·5	94	110	154	144

Dog H (*Dog with Bickel Pouch*).—The secretory response of the Bickel (denervated) pouch to a meal consisting of 100 g. of meat mixed with 200 c.c. of water was much less affected by previous introduction of acetic acid into the main stomach than was that of a normally innervated stomach or pouch. The variations in the volume of fluid and of mucus and in the different constituents of the juice (fig. 3)

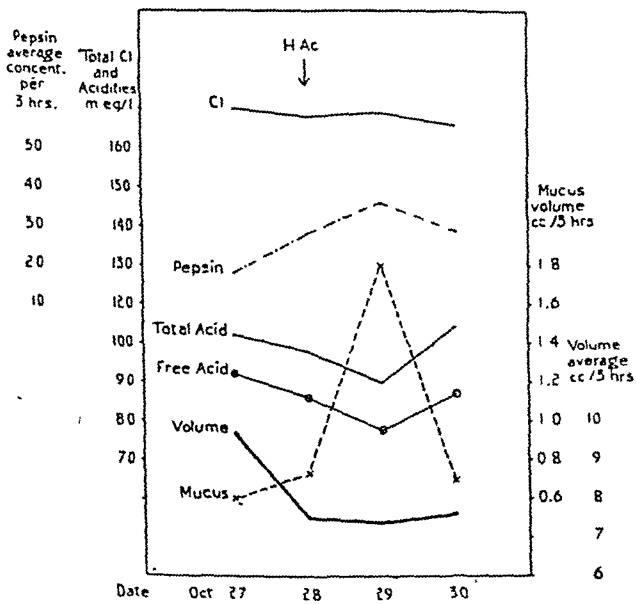

FIG. 3.—The effect of previous introduction of acetic acid solution into the main stomach on the secretory response of a Bickel pouch to a test-meal in a dog.

with the highest concentration of mucus in the juice (Oct. 4, 7·4 per cent.; Oct. 5, 18·5 per cent.; Oct. 6, 20·0 per cent.; and Oct. 7, 5·5 per cent.), although the total volume of mucus on Oct. 6 was somewhat less than on the day that acetic acid was administered.

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Date.	Volume, c.c.	Free acidity, m.eq./l.	Total acidity, m.eq./l.	Mucus content, per cent.	Remarks.
Oct. 4	35·0	121	137	8·1	
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mucus by the gastric mucous membrane in response to the application of acetic acid solution.

Already one of us [Babkin, 1929] has emphasized the importance of the part played by the mucous cells of the neck in the regulation of the acidity of the gastric juice. Hollander [1934] called the secretion of all the cells of the gastric glands other than the acid-producing cells a "diluting secretion," or "alkaline component," and supposed it to have a chloride concentration of about 100 m.eq. per litre—that is, lower than that of the acid secretion of the parietal cells. Some clinicians—Kalk [1932], Katsch [1933], Maclagan [1934], and others—consider that the level of the acidity of the gastric juice depends on the activity of the "mucous cells of the neck," which produce a neutral, watery secretion. To what extent the diminution of the acidity after the administration of acetic acid was due to actual neutralization of the hydrochloric acid of the gastric juice, and to what extent to dilution by excessive secretion of the surface epithelium cells and mucous cells of the neck, we are unable to say. It is true that in many of our experiments the activity of the parietal cells was diminished, as evidenced by the diminished volume of the gastric secretion, and the activity of the mucous and mucoid cells increased. In other experiments, in spite of a practically normal secretion of visible mucus, both the free and the total acidity were low. However, this fact cannot be explained by Rosemann's [1907] theory that the parietal cells are capable of producing hydrochloric acid in varying concentrations, because in our experiments with diminution of the acidity of the juice the concentration of total chloride likewise diminished (*cf.*, for example, the data in Table III.). This fact refutes one of the fundamental postulates of Rosemann's theory, namely, that the concentration of total chloride in the gastric juice remains constant, but that the relative amount of chloride combined with *H* ion or with *Na* ion varies. It seems to point to the admixture of the parietal secretion with a secretion possessing a lower chloride concentration.

One other point has to be mentioned. The data here presented are not in agreement with the "diffusion theory" of Teorell [1933, 1935, 1939, 1940] concerning the regulation of the gastric acidity. According to Teorell the hydrochloric acid of the gastric juice is diffused into the gastric mucosa or the blood and is exchanged against sodium chloride *i.e. Na* ions replace the *H* ions and neutral chloride is formed. The theory was based on experiments on cats, in which a very small amount (usually 3 to 5 c.c.—maximum 10 c.c.) of an acid of known concentration was introduced into the stomach, and samples of gastric juice were taken for chemical analysis every 15 minutes for 1½ hours. We do not dispute Teorell's facts. We consider, however, that the conditions necessary for the exchange of the ions of an acid solution which is present in the stomach occur rarely under normal

seem to indicate that the acetic acid after it had left the stomach still affected somewhat the secretion of the isolated pouch. However, it is to be noted (1) that these variations were slight; (2) that in many control experiments the volume of secretion and especially the volume of mucus, which is abundantly produced by this kind of pouch, underwent analogous variations; and (3) that the figures for pepsin concentration in the Bickel-pouch juice are in general so small that the observed variations signify very little. Neverthless one fact is clear, namely, that in this type of experiment the lowest free and total acidities coincided with the highest content of mucus in the juice.

Unfortunately, as there was no other dog in the laboratory with a Bickel pouch and a gastric fistula, this type of experiment could not be repeated on another animal.

Discussion.

The experiments we have reported confirmed the observation of Gorbunova, Lebedinskaïa and Savich [1933] that the introduction of acetic acid into the main stomach affects the character of the gastric secretion of a Pavlov pouch. They also indicated clearly that the secretory activity of different histological components of the gastric mucosa must receive due consideration in any discussion of the regulation of the acidity of the gastric juice. The introduction of acetic acid solution into the stomach was used as a means of stimulating chiefly one group of epithelial cells of the gastric mucosa, namely, those producing mucus or a mucoid secretion. Acetic acid, whether acting directly on the mucosa or at a distance, produced a secretion of mucus greater than normal, which was accompanied by a diminution in the acidity of the gastric juice and in most cases by a slight diminution in the total chloride concentration. The lowering of the acidity was due not to the diminished rate of secretion but to the increased mucus content of the juice, as may be seen from fig. 1 (Nov. 25 and 26—sham-feeding with meat) and also from Table IV. (Feb. 2 and 4—rectal administration of alcohol). By "mucus" we do not mean only visible mucus in the form of lumps and threads, which could be centrifuged, and the amount of which merely serves as an indication of the changes in the production of the "mucoid secretion." It is very probable that not only is a certain amount of neutral or alkaline fluid secreted by the mucous cells of the gastric glands along with the gastric "mucus," as was shown by Florey [1931] to occur in the case of the colonic mucus, but there is another large group of cells in the gastric mucosa, namely, the mucous (or chief) cells of the neck, which also are able to produce a neutral or alkaline mucoid secretion. Integrity of the vagal innervation of the stomach seems to be an important factor in the increased production of

a much lesser degree in a Bickel-pouch dog, the introduction of acetic acid into the main stomach in these cases influencing the secretory activity of the pouch *par distance*.

3. Evidence is advanced that the lowering of the acidity and of the total chloride concentration of the gastric juice in these experiments was due to the neutralizing and diluting effect of the mucoid secretion, an excessive production of which was caused by the introduction of acetic acid into the stomach.

We are greatly indebted to Mrs. Olga Komarov for her help in a number of these experiments.

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circumstances. They may occur perhaps when an exceedingly small amount of gastric juice remains for some reason in a resting or very feebly secreting stomach for a considerable time. However, Teorell's explanation, if accepted *in toto*, implies that gastric mucus possesses no acid-binding capacity, which does not agree with the data obtained in this investigation. In our experiments the gastric juice never stagnated either in the whole stomach or in a pouch, but was able to flow freely through the fistula. Thus, for instance, in the sham-feeding experiments, both on the day of administration of acetic acid and on the day after (fig. 1—Nov. 25 and 26) the rate of secretion was the same, being more than 60 c.c. per hour, yet the total and the free acidity and the chloride concentration of the juice differed greatly on the two days. Tables I. and II. show that in some of the experiments in which acetic acid had been administered the volume of the gastric secretion was greater than in the control experiments, and yet the acidity of the juice was lower in the former than in the latter. To explain this phenomenon according to the "diffusion theory," the fact of the greater secretion of mucoid fluid must be completely ignored and an additional hypothesis must be introduced, namely, that the acetic acid increased enormously the ability of the gastric mucosa to exchange the ions of the juice. Even if this assumption were correct, it would not provide an adequate explanation for many of our experiments. Thus, for example, in the Pavlov-pouch dog, in which the acetic acid did not come in direct contact with the pouch mucosa, the greatest depression of the activity occurred not on the actual day of administration of the acid but on the following day, when the mucus content was high and the total chloride concentration was particularly low (see fig. 2).

The problem of the regulation of the gastric acidity is a complicated one, as the present experiments plainly show. For the study of this problem it is essential to have a thorough understanding of the secretory activity of the various cellular groups which form the gastric glands and mucosa, including a knowledge of the composition of the secretion from each group of glandular cells, and of the mode or modes of regulation of the secretory process in each group.

Summary.

1. In dogs with œsophagotomy and a gastric fistula, 1 per cent. acetic acid solution, introduced into the stomach and kept there for 2 to 2½ hours, greatly affected the secretory response to subsequent sham-feeding with meat or subcutaneous injection of histamine, diminishing the volume of the gastric secretion, lowering the total chloride concentration and the free and total acidities, and increasing the content of mucus.

2. Analogous results were obtained in a Pavlov-pouch dog and in

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The view that absorption of toxic substances from damaged tissues, particularly muscle, caused shock received prominence as the result of clinical and experimental evidence collected during the last war. It was suggested that a substance, either histamine or something similar, might be liberated from the tissues by injury and absorbed into the blood stream, causing general capillary damage and subsequent circulatory collapse [Cannon, 1923]. Since that time doubt has been cast on the interpretation put on the experimental and clinical observations.

It has further been suggested that during tissue autolysis substances might be liberated which could produce a shock-like state when absorbed into the blood stream. Moon [1938], summarising the experimental evidence, concluded that there were good grounds for the conception of a toxic cause of shock, and one of his postulates was that autolysing tissues liberated some substance causing circulatory collapse. Mason and Davidson [1924–25 *a* and *b*], having found that crude heparin preparations were toxic to man, investigated the toxicity of liver to dogs and discovered that when portions of liver were excised and left in the abdominal cavity a shock-like state was produced and death supervened in 15 to 20 hours. They obtained similar results with pieces of spleen [1924–25 *c*]. At post-mortem they noted that the implanted liver was chocolate colour and full of gas, but they did not make bacteriological investigations. Later [1924–25 *b*] they showed that saline extracts of fresh tissues were toxic when injected intravenously and that a similar extract of liver "autolysed" *in vivo* in the peritoneal cavity of one dog was toxic to another.

Ellis and Dragstedt [1930] repeated the implantation experiments and confirmed the findings of Mason and Davidson, but they regarded the condition as a bacterial toxæmia rather than a toxæmia due to the absorption of autolytic products. They pointed out that most workers had been quite unable to obtain adult tissues (usually liver) free from bacteria, and that the organisms appeared to be normal saprophytes. Accordingly, they implanted fœtal livers obtained by Cæsarean section and here they found no toxic effects, although the eventual absorption

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of the pieces of liver was evidence that autolysis had occurred. Mason and Lemon [*1932*] freely admitted the presence of a gram-positive sporing organism in liver pulp, but thought nevertheless that it was an autolytic product from the liver which caused death, by inducing a chemical peritonitis and excessive loss of fluid into the peritoneal cavity. With Nau, Mason [1935] again presented the "autolytic" rather than the "bacterial" toxin as the factor responsible on the evidence that cultures of the liver organism could be injected without causing symptoms, but since death or survival ran parallel with the presence or absence of organisms he admitted that bacterial action might accelerate the production of "autolytic" toxin. Moon [*1937*] found that 5 g. per kilo of minced muscle intraperitoneally was lethal to dogs, but did not mention whether bacteria were found post-mortem. He later [*1938, p. 280*] *found that from 1 to 3 g. per kilo of liver killed* 6 out of 10 guinea-pigs in 5 to 14 days, while larger doses of 3 to 5 g. per kilo killed all of 25 animals in a few days. On the other hand, *Parsons and Phemister [1930] excised the right rectus abdominis* muscle from 3 dogs, and returned it to its sheath in 1 and to the abdominal cavity in 2 without producing any toxic symptoms.

Andrews and Hrdina [1931] implanted pulped liver but, as a preliminary, they incubated it for 24 hours and then autoclaved it for 40 minutes. Death ensued, and large numbers of gram-positive anaerobic bacilli were present in the pulp at post-mortem, while the abdominal cavity contained gas and much free fluid. This result was obtained in both dogs and rabbits. In a second series of experiments fresh liver was pulped and autoclaved and inserted immediately; 4 out of 6 animals died with similar bacteriological findings. An intraperitoneal injection of 30 c.c. of a litmus milk culture of *Cl. welchii*, however, did not kill, and the peritoneal cavity was sterile 4 days later. The authors suggested that a substance liberated from liver pulp increased the permeability of the gut wall to organisms. Dvorak [1931], after a long series of similar experiments on dogs, concluded that death was due to bacterial action and not to the autolysis of the implanted liver. Saltzmann [1932] claimed that ground-up liver put into the peritoneal cavity of the rat caused peritonitis but did not kill, although bacteria were present *in the implant and in the host's liver. Auto*claved liver did not produce any reaction. Two dogs were killed in 6 and 16 hours respectively by 100 g. of liver, and here the bacteriological cultures were sterile. Saltzmann did not agree with Andrews and Hrdina that bacteria emerged from the gut, but thought that death was caused by the absorption of some toxic protein autolysate—an "albumose." Trusler, Reeves, and Martin [1935] inserted liver autoclaved on excision and after various periods of incubation. They took their results to indicate that death of the animals was associated with concomitant or previous bacterial contamination of the implanted

liver or autolysate even when the peritoneal cavity was sterile at postmortem. They found it necessary to practise excessive, as opposed to ordinary, surgical asepsis in order to avoid contamination from the skin or muscles of the abdominal wall. They obtained no evidence that death from "chemical" peritonitis, whether produced by incubated and autoclaved tissues or, for example, by bile salts, was associated with increased passage of bacteria from the intestine to the peritoneum. Boyce and McFetridge [1937] gave a good review of previous work and repeated much of it with great care. Ellis and Dragstedt's experiments with fœtal liver were only confirmed for small amounts—35 g. had no effect in the peritoneal cavity of a dog, but 70 g. caused illness, and 150 g. death in 24 hours. Unfortunately no bacteriological tests were performed in this experiment; nevertheless, in most of those that they record, liver implantation was associated with the presence of gas-producing anaerobic organisms. They were of the opinion that death of their dogs was associated with autolytic products, but that gas bacillus infection was an important additional factor.

Wolbach and Saiki [1909] demonstrated that bacteria could often be grown from dogs' livers (21 out of 23 dogs). Reith [1926] exhaustively reviewed the literature on organisms in living tissues and confirmed by his own work their frequent presence in three different species. Berg, Zau, and Jobling [1926–27] found the same organism as Wolbach and Saiki. Gage [1930–31] found a gram-negative spore-bearing bacillus in 34 out of 40 dogs' livers, 6 having gram-positive organisms in addition. Trusler and Reeves [1934] found gram-positive anaerobic organisms in the liver and muscle of healthy dogs, not *Cl. welchii* and not exotoxin producers. Recently Mason and Hart [1939–40] have described a "Welch-like bacillus" in the human liver. Wilson and Roome [1936] noted that the muscles in dogs' legs, both normal and after constriction by a tourniquet for some hours, contained an anaerobic gas-forming bacillus.

While there is universal agreement that bacteria can be cultivated from various tissues—the liver in particular—even when the strictest possible bacteriological technique is employed for obtaining the tissues, there is little concurrence as to the type of organism, except the negative one that it is probably not *Cl. welchii*. The difficulty in interpreting the numerous experiments on autolysis of liver and other tissues *in vivo* resides in that of assessing the importance of these gas-producing anaerobes. Their importance might lie firstly in producing a toxin, and secondly in producing powerful enzymes, especially proteases, more active than autolytic enzymes in breaking up liver tissue. That such enzymes do in fact come from these organisms was shown by Jackson [1909 *a* and *b*] when he investigated chemical changes in excised livers—a sterile liver decomposed much more slowly than a contaminated one.

According to some French authors there is an increase in the polypeptides of the blood following trauma and burns [Fiessinger, 1934, who reviews the French literature, and Duval, Roux, and Goiffon, 1934]. They consider these polypeptides to be toxic, but produce no evidence that this is so. Étienne-Martin [1938] considers that there is no evidence that the polypeptides are toxic, and quotes his own and some Italian experiments, to which he refers in support of this. He considers it possible that lipid fractions of autolysis may be toxic.

It appeared to us desirable to determine whether some of the conflicting evidence could be resolved, and if possible to establish whether autolysis in the absence of bacterial contamination can cause death with shock-like changes.

Experimental.

The great majority of previous experiments have been done by implanting material in the peritoneal cavity, and it might be objected that the absorption into the blood stream of toxins was considerably less effective from this cavity than from, say, damaged muscle. However, the lymphatic absorption from the peritoneal cavity is particularly effective through the lymphatics of the diaphragm, which are freely permeable even to such large particles as blood corpuscles. In addition, direct absorption into the blood stream is considerable, so that if death is not caused by the presence of toxins in the peritoneal cavity it is not likely to occur from their presence, in similar amounts, in damaged muscle.

In most of our experiments, therefore, intraperitoneal transplantation was done on rabbits, and on some rats, cats, and guinea-pigs.

Aseptic precautions such as those usually employed in surgical procedures were taken, gloves and masks being worn, and all instruments, cloths, etc., carefully sterilised.

In the first series liver and leg muscle was removed from rabbits anæsthetised with ether. The tissue was usually ground very finely in a sterile Latapie mincer before insertion into the peritoneal cavity of the host. During this procedure the material was exposed to air, and during the actual insertion with a spatula it touched the skin of the host. Dosage was measured in c.c. of the pulped tissue. It was soon apparent that if a sufficient dose of liver or muscle was inserted the host died, but that in the dead animals much gas was found in the autolysing tissues, and considerable amounts of blood-stained fluid were always present in the peritoneal cavity. Table I. gives a résumé of the results.

A second series was done in which 4 rabbits were given a dry diet before and after operation and 3 given greens *ad lib.* before and after operation. Red-cell counts were made on blood from an ear vein. It

will be seen from Table II. that a considerable hæmoconcentration occurred, though one animal with a hæmoconcentration of +62 per cent. and another with +44 per cent. survived.

TABLE I.—INSERTION OF CHOPPED TISSUE INTO THE PERITONEAL CAVITY.

Rabbit no.	Tissue inserted.	Dose per kilo, c.c.	Hours before death, at least.	P.M. Bact. or gas.
6	Liver.	5	18	+
7	,,	2·5	24	+
8	,,	1·25	Survived.	
9	Muscle.	5	,,	
10	,,	2·5	,,	
11	,,	1·25	,,	
24	Liver.	10	29	
15	,,	4·3	21	
16	,,	2	Survived.	
25	Muscle.	25	21	+
17	,,	9	Survived.	
18	,,	4	,,	
44	Liver.	10	15	+
45	Muscle.	20	15	+

TABLE II.—DRY DIET AFTER OPERATION.

Rabbit no.	Tissue inserted.	Dose per kilo, c.c.	R.B.C., per cent.	Hours before death, at least.	P.M. Bact. or gas.
27	Liver.	10	+40	20	+
28	,,	10	+34	20	+
29	Muscle.	20	+20	21	+
30	,,	20	+12	21	+
Greens ad lib. after operation.					
33	Liver.	10	+62	Survived.	
34	Muscle.	20	+44	,,	
35	,,	20	..	17	+

In the next series of animals boiled or autoclaved minced tissue was inserted. Though the result was not completely clear-cut there was evidently a better survival rate than when the tissues were not heated. It is possible that the sterilisation was not effective in every case (Table III.).

A series of experiments was then carried out in which the minced tissue was placed in cellophane tubes. The sacs had a diameter of 1·75 cm. and were about 12–14 cm. long. The ground material was

placed in them, both ends being tied off securely so that no leakage occurred. The object of this procedure was to determine whether any toxic substance of small molecular size was given off from the autolysing tissue and to try to keep any bacterial growth confined to the inside of the sac. As was subsequently shown, the precautions against bacterial contamination were at first not adequate in these experiments.

TABLE III.—INSERTION OF BOILED OR AUTOCLAVED MINCED TISSUE.

Rabbit no.	Tissue inserted.	Dose per kilo, c.c.	Hours before death, at least.	P.M. Bact. or gas.
19	Liver.	7	Survived.	
20	Muscle.	8	"	
38	Liver.	10	Killed—peritonitis.	+
39	"	12	Survived.	
40	Muscle.	23	20	
41	"	21	Survived.	

In the first two experiments when death occurred the sacs were tightly blown up with gas, and free fluid contaminated with bacteria filled the peritoneal cavity (Table IV.).

TABLE IV.—INSERTION OF TISSUE IN CELLOPHANE SACS.

Rabbit no.	Tissue inserted.	Dose per kilo, c.c.	Hours before death, at least.	P.M. Bact. or gas.
52	3 sacs muscle.	20	16½	+
53	2 sacs liver.	10	Moribund and killed in 18 hours.	+

In the next series toluene was added to the material in the sacs in the hope of keeping down the bacterial growth. Precautions were also taken to prevent the external surface of the sac being soiled in any way, and it was protected by a glass tube during its insertion into the peritoneal cavity. As a preliminary two rabbits each had 2 sacs implanted, containing in one rabbit 45 c.c. saline and 0·5 c.c. toluene and in the other 35 c.c. saline and 1·0 c.c. toluene. These rabbits exhibited no disturbance whatever.

From Table V. it will be seen that all but one of 6 rabbits survived. The sacs of the rabbit which died were full of gas and many bacilli were present and there was free fluid in the peritoneal cavity.

Several days after implantation the remainder of the sacs were removed and found to be flaccid, no gas having developed in them.

On the other hand, autolysis, judged from the appearance of the minced liver and muscle, was not advanced.

Experiments were therefore done to determine the effect of toluene and other antiseptics on liver autolysis.

For experiments with toluene and chloroform a rabbit liver was extracted and handled with the usual precautions to avoid contamination. The liver was ground in a Potter and Elvehjem "Homogeniser" [1936], and portions of the fine "brei" were pipetted into sterile bottles.

TABLE V.—INSERTION OF SACS CONTAINING SALINE AND TOLUENE.

Rabbit no.	Tissue inserted.	Hours before death, at least.
56	2 sacs each with 45 c.c. saline + 0·5 c.c. toluene.	Survived.
57	2 sacs each with 35 c.c. saline + 1 c.c. toluene.	"

INSERTION OF TISSUE AND TOLUENE IN SACS.

Rabbit no.	Tissue inserted.	Dose per kilo, c.c.	Hours before death, at least.
59	2 sacs muscle.	30	Survived.
60	" "	30	"
61	" "	30	"
62	" "	27 (Sac full of bacilli P.M.)	18
63	2 sacs liver.	12	Survived.
64	" "	12	"

An equal volume of 10 per cent. trichloracetic acid was added immediately to one of these, as a control. The others were incubated with shaking, alone and in the presence of toluene or chloroform, for 16 hours. There was no evidence of gas production in any of the bottles, and the contents were sterile. Only in the case of the material incubated without toluene or chloroform was there noticeable liquefaction. These samples were then also mixed with an equal volume of 10 per cent. trichloracetic acid and centrifuged together with the control. The increase in amino nitrogen on incubation, determined in 1 c.c. portions of the filtrates by the method of Van Slyke, was taken as a measure of the relative extent of autolysis. The results, which are summarised in Table VI., show that in the presence of toluene the amount of autolysis was about 50 per cent., and in the presence of chloroform only about 10 per cent. of that of the untreated liver.

It was subsequently found that Jackson [1909 *b*] had observed great

slowing of autolysis when toluene or chloroform was added to liver, and the use of toluene for the purpose of ensuring the absence of bacterial action was made even less attractive through the observation of Miss R. Schoental that growth of *Vibrion septique* occurred in normal broth, containing ascorbic acid, which had been covered by a layer of toluene.

TABLE VI.

Sample.	Mg. amino N_2 per c.c. liver.	Increase on incubation mg. amino N_2.
1. Fresh liver	1·3	..
2. 5 c.c. liver + 0·5 c.c. toluene incubated 16 hours.	2·2	0·9
3. 5 c.c. liver + 0·1 c.c. toluene incubated 16 hours.	2·3	1·0
4. 5 c.c. liver + 0·5 c.c. chloroform incubated 16 hours.	1·5	0·2
5. 5 c.c. liver incubated 16 hours . .	3·2	1·9

Attempts were then made to find an antiseptic which would allow liver autolysis to proceed normally while suppressing the anaerobes whose presence had been found to be associated, in the transplantation experiments with rabbits, with the death of the animal. Quinanil (1 : 1000) and merthiolate (1 : 5000) were found to be unsatisfactory. After 18 hours incubation in their presence, samples of liver which had been handled without aseptic precautions were heavily infected with gas-producing organisms. The following experiments, however, showed that acriflavine was a suitable substance. A rabbit liver was removed without aseptic precautions and homogenised. Portions were then incubated in test tubes, alone and in the presence of acriflavine (1 : 1000 and 1 : 2000) for 44 hours. The tubes were then set up in duplicate and the samples incubated both aerobically and anaerobically. In the tubes without acriflavine there was considerable production of gas and a characteristic repulsive smell, but not in the others. An examination of smears of the contents of the tubes showed that *welchii*-like organisms had grown profusely in both tubes having no acriflavine, but were absent from the others. All the latter contained streptococci, but these were scanty in the tubes with the higher concentration of acriflavine. The amount of autolysis under different conditions was measured, by the method of Van Slyke, as previously described, and the results are given in Table VII. The figures show that considerable autolysis occurred in the presence of acriflavine, when the growth of anaerobes had been suppressed. The amount of autolysis was similar

under aerobic conditions. It was about 75 per cent. of that of untreated infected liver when the latter had been incubated anaerobically and 90 per cent. when the incubation was aerobic.

TABLE VII.

Sample.	Incubation.	Mg. amino N_2 per c.c. liver.	Increase on incubation mg. amino N_2.
1. 5 c.c. fresh liver	..	1·1	..
2. 5 c.c. fresh liver + 0·5 c.c. acriflavine	..	1·1	..
3. 5 c.c. liver + 0·5 c.c. acriflavine (1 : 100)	Aerobic.	7·4	6·3
4. 5 c.c. liver + 0·25 c.c. acriflavine (1 : 100)	,,	7·2	6·1
5. 5 c.c. liver + 0·5 c.c. acriflavine (1 : 100)	Anaerobic.	7·2	6·1
6. 5 c.c. liver + 0·25 c.c. acriflavine (1 : 100)	,,	6·8	5·7
7. 5 c.c. liver	Aerobic.	7·7	6·6
8. 5 c.c. liver	Anaerobic.	9·2	8·1

Table VIII. gives the results of injecting liver digests made *in vitro*, with and without the addition of acriflavine. No precautions were taken to exclude bacteria during digestion. The digests were filtered through a Seitz filter just before injection into the peritoneal cavity. All the animals receiving acriflavine digests survived, while 2 out of 3 receiving the "plain" digest died quickly with gas present in the peritoneal cavity, and the third died after 5 days from what appeared to be tetanus.

TABLE VIII.—INTRAPERITONEAL INJECTION OF AUTOLYSATES OF LIVER.

Rabbit no.	Tissue inserted.	Dose per kilo, c.c.	Autolysed.	Hours before death, at least.
86	Seitz-filtered, acriflavine.	75	40 hrs.	Survived.
87	,, ,, plain.	75		,,
88	,, ,, plain.	70	24 hrs.	Died 15 hrs.
89	,, ,, acriflavine.	70		Survived.
90	,, ,, acriflavine.	60	24 hrs.	,,
91	,, ,, plain.	60		Killed 18 hrs.

Menkin [1936, 1938 *a*, *b*, *c*] noted that pus had in it a substance "leukotaxin" which caused increased permeability of blood capillaries. Duthie and Chain [1939] found that fibrin and also a number of purified proteins digested by pepsin contained a polypeptide which had also this permeability increasing property. Of all proteins tested, gelatine digests were found to contain least of this substance. It is probable that such a polypeptide is liberated during autolysis.

A further series of experiments was therefore done (Table IX.) in which large amounts of peptic digests of fibrin and gelatine were injected. These digests were made as follows: 20 g. of cattle fibrin (B.D.H.) or gelatin was mixed with 200 c.c. of water (the gelatine being dissolved by warming) and the mixture brought to pH 2·5 by the addition of 10 N HCl. 740 mg. of Park Davis pepsin (1 : 10,000) was then added and digestion carried out by shaking in the incubator for 8 hours. The solution was brought to pH 5 with NaOH, made isotonic if necessary by the addition of NaCl, and centrifuged. The pH was then adjusted to 7·4 and the solution filtered. Digests made in this manner contained about 85 mg. of solid material per c.c. Some of the fibrin digests were tested for their content of permeability factor by Dr. E. S. Duthie [*cf.* Duthie and Chain, 1939]. They gave positive permeability tests in the rabbit down to dilutions of 1 : 100.

TABLE IX.—INTRAPERITONEAL INJECTION OF SEITZ-FILTERED DIGESTS.

Rabbit no.	Material inserted.	Dose per kilo, c.c.	Hours before death, at least.	P.M. Bact. or gas.
78	Pepsin digest of fibrin.	70	Killed 18 hrs.	Sterile.
79	,, ,, gelatin.	60	Survived.	
80	,, ,, ,,	60	,,	
81	,, ,, ,,	50	,,	
82	,, ,, fibrin.	70	,,	
83	,, ,, ,,	40	,,	
84	,, ,, ,,	20	,,	
85	,, ,, ,,	10	,,	
71	,, ,, ,,	70	Died 17 hrs.	
72	,, ,, ,,	100	Survived.	
76	,, ,, ,,	45	,,	

The injected fluid became contaminated in some of the earliest experiments of this type though inadequate sterile precautions seemed to have been taken. In the experiments of which the results are recorded (Table IX.) the fluid was led straight from a Seitz filter through a sterile glass tube into a small hole in the belly wall. This hole had a purse string suture placed round it in the muscle layer before the glass tube was inserted; the suture was tied round the glass tube during injection and tightened as the tube was removed. It was hoped thus to keep the peritoneal cavity sealed throughout the operation. It will be noted that a sterile digest only causes death very occasionally. Three experiments were also done in which the digest was inserted in cellophane bags. One of these animals was killed when dying at 63 hours (Table X.). The amount of autolytic proteolysis taking place in sterile liver during 24 hours is small, even under favourable conditions, and that of muscle, the damage of which is generally held to be

particularly associated with wound shock, is much smaller still [Bradley, 1938]. In view of these facts, and the failure of the large quantities of concentrated fibrin digest injected to produce shock-like symptoms, it seems unlikely that a permeability-increasing polypeptide resulting from autolytic digestion of tissues can significantly increase the capillary permeability after absorption. Its effect in increasing capillary permeability is purely local. Duthie and Chain [1939] found that intravenous injection of 5 c.c. of a 5 per cent. solution of active permeability-increasing polypeptide fractions had no effect on the hæmoglobin level in rabbits over a period of 5 hours.

Table X.—Insertion of Digests and Dialysates in Sacs.

Rabbit no.	Material inserted.	Dose per kilo, c.c.	Hours before death, at least.	P.M. Bact. or gas.
74	Pepsin digest of fibrin.	80	Survived.	
75	,, ,, gelatin.	30	,,	
77	,, ,, fibrin.	75	63	Sterile.

During the course of certain of the liver-autolysis experiments bacteriological examination showed that rabbits' liver could sometimes be procured sterile and that it then remained sterile during incubation. It seemed to us therefore that, provided our technique was sufficiently rigid, we should be able to transplant ground-up rabbit's liver without bacterial contamination.

A further series was therefore done using the following procedures.

After shaving the belly and lower thorax the donor animal was anæsthetised with ether and the shaved area thoroughly treated with a solution of 4-chloro-3 : 5 xylen-1-ol in 50 per cent. alcohol. This substance is a good disinfectant and kills bacterial spores very rapidly. A cotton-wool pad was then soaked in the fluid and left applied to the abdomen and lower thorax. The animal was bled to death from the carotid artery and all the fur thoroughly damped down with the chlorxylenol solution. The rabbit, which was tied to a board, was covered with a sterile cloth and transported to a clean operating theatre. With scrupulous care the liver was removed and immediately covered. After preliminary chopping with scissors the liver was put into a sterile Latapie mincer. Great care was taken to prevent organisms dropping on to the liver during this manœuvre. The ground liver, as it emerged from the mincer, was received directly into a glass syringe with a wide exit. The syringe was protected by an outer glass vessel and by a protective "frill" at the upper end to avoid external contamination. The plunger of the syringe was inserted and the air expelled from the syringe. The syringe was left com-

pletely covered in a sterile glass tube while a small incision was made in the belly wall of a "host" rabbit whose abdomen had also been prepared as described. A purse-string suture was placed around the muscle opening and the mouth of the syringe tied into it. The dose of liver was delivered from the syringe and the abdomen closed at the moment of withdrawal of the syringe by pulling the purse-string suture tight. A skin suture was inserted and the abdomen was massaged to spread the liver in the peritoneal cavity.

Cultures were taken from the material left in the syringe.

Table XI. gives the results. It will be seen that 4 out of 5 animals survived and that the animal that died was infected in spite of all

TABLE XI.—INTRAPERITONEAL LIVER WITH VERY RIGID ASEPTIC PRECAUTIONS.

Rabbit no.	Tissue inserted.	Dose per kilo, c.c.	R.B.C., per cent.	Hours before death, *at least.*	Bacteriology of tissue *inserted.*
92	Minced liver.	10	Not done.	Survived.	Sterile.
93	,, ,,	*12·5*	*+28*	*Killed 23 hrs.*	*Infected.*
94	,, ,,	11	+33	Survived.	Sterile.
95	,, ,,	11	+33	,,	,,
96	,, ,,	11	+22	,,	,,

our precautions. It is instructive to note that all the animals in which the examination was done exhibited very marked hæmoconcentration, though, with the exception of the one which died, they remained apparently in good condition and after a few hours took their food normally. There did not seem to be any difference with regard to hæmoconcentration between the animals which died and those which did not. *Earlier in this series of experiments, before the necessity for* all the precautions above outlined were realised, rabbits had been prepared with carotid loops by van Leersum's method so that blood-pressure records could be made on the unanæthetised animal. These animals were "trained" over a period of at least 14 days by the frequent taking of blood-pressure readings by a technique which has been found satisfactory in other experiments dealing with hypertension. The "normal" pressure was thus determined and steady readings obtained for several days before the operation of liver implantation. The 4 animals submitted to experiment died in from 10 to 18 hours with gross infection (gas production) in the peritoneal cavity. They all showed hæmoconcentration, but there was only a slight fall of blood-pressure till shortly before death, though one animal had rather a low pressure after the operation of liver insertion (see Table XII. and figs. 1 to 4).

In rabbit 12 at $7\frac{1}{2}$ hours after liver implantation the usual pressor response to excitement was noted—the pressure rising to 140 mm. Hg.

It was noticed that the animals which died had collections of blood-stained fluid in the peritoneal cavity and small amounts of fluid which was sometimes clear in the pleural cavities. The congestive changes in various organs of dogs described by Moon [1938] were rarely seen in our rabbits. There was reddening of the peritoneal contents where contact with the implant or its fluid contents occurred. The lungs rarely showed any significant change though blood-stained fluid was found in the pleural cavities. Sometimes œdema of the mediastinum with the presence of gas bubbles was encountered.

Table XII.—Insertion of Minced Tissue; Blood Counts and Blood Pressure Records made.

Rabbit no.	Tissue inserted.	Dose per kilo, c.c.	B.P.	R.B.C., per cent.	Hours before death, at least.	P.M. Bact. or gas.
3	Muscle.	20	No fall.	+25	14	+
4	Liver.	10	Terminal fall.	+30	18	+
12	Muscle.	25	No fall.	+30	10	+
14	Liver.	11	Low after operation.	+37	12	+

It appeared to us probable that the hæmoconcentration could be explained adequately by this local loss of fluid without having to postulate the absorption of a toxin into the general blood stream.

It has already been noted that polypeptides which are known to increase capillary permeability are produced during digestive breakdown of tissues. It may be objected that fluid appeared in the pleural cavity which did not contain autolysing tissue or digest. As has been pointed out, absorption from the peritoneal cavity by the diaphragmatic lymph-capillaries is particularly free. This absorbed material could quite readily escape from the pleural lymphatic capillaries and cause a localised increase of permeability in the neighbouring blood capillaries.

The permeability of the blood capillaries to pontamine blue was tested on three rabbits which had tissues placed in the peritoneal cavity. After some hours interval pontamine blue, which escapes from normal blood-vessels very slowly, was injected intravenously. The dye was found to escape from the blood-vessels in the peritoneal cavity only in the neighbourhood of the implanted tissues and was particularly intense where the tissues were in contact with the intestines. No generalised increase of permeability was discovered in any other tissues.

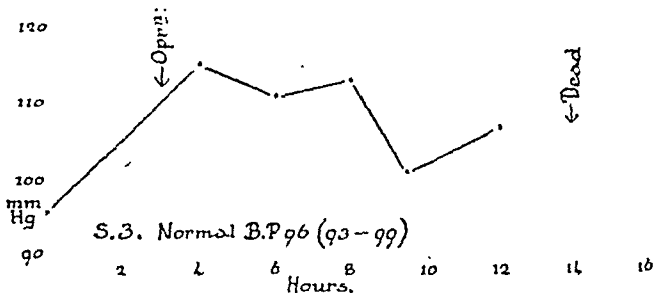

FIG. 1.

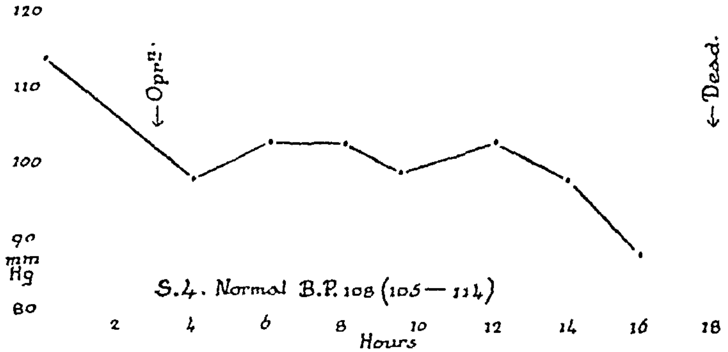

FIG. 2.

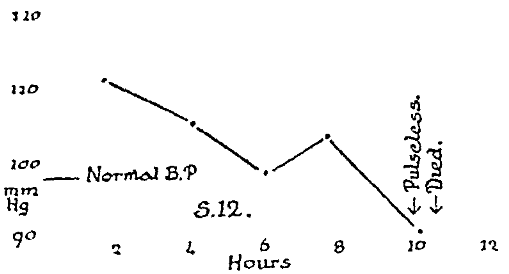

FIG. 3.

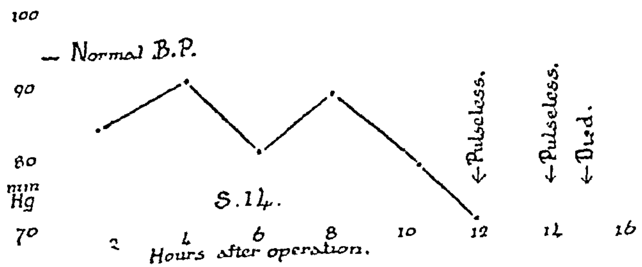

FIG. 4.

In the one animal in which the examination was made there was a 27 per cent. increase in the red-cell corpuscles (Table XIII.).

Bacteriological examination showed gross contamination of the peritoneal contents.

TABLE XIII.—INSERTION OF TISSUE. KILLED AFTER PONTAMINE INJECTION.

Rabbit no.	Tissue inserted.	Dose per kilo, c.c.	R.B.C., per cent.	Killed. hrs.	P.M. Bact. or gas.
54	Liver.	10	..	8½	
55	Muscle.	20	..	8½	
97	Minced liver.	14	+27	11½	+

Caution has to be exercised in the interpretation of hæmo-concentration in rabbits, for simple preparation for operation and ether anæsthetisation were found to give the following figures: Rabbit, weight 2285 g., anæsthetised with ether and kept on board for ¾ hour. Prepared for operation. Chlorxylenol applied to abdomen for 10 minutes.

Preoperative red-cell count . .	6·51 million.
3 hours post-operatively . . .	8·28 ,,
23 hours post-operatively . .	6·40 ,,

On the other hand, another animal treated in exactly the same way gave this result: Rabbit, weight 2725 g. ¾ hour anæsthesia, etc. as in last experiment.

Preoperative red-cell count . .	6·20 million.
3 hours 50 minutes post-operatively .	5·93 ,,
6 hours 40 minutes post-operatively .	6·01 ,,

This question was not pursued further, but it is of interest that McAllister [1937] reported the reduction of plasma volume following ether anæsthesia. While Cannon [1923, p. 164] reports work by Mann and by Epstein to the same effect.

The bacteriological investigations regularly disclosed in contaminated specimens a gram-positive anaerobic rod. Such an organism was present also in contaminated peptic digests so that it did not necessarily come from the ground-up liver or muscle. No attempt was made to identify this organism. In addition, various aerobic cocci, again not identified, were frequently found. That this bacterial contamination was associated with an inadequate operative technique was eventually shown. In the rabbit it is possible to get sterile liver mince when elaborate precautions are taken.

In addition to these several groups of experiments done on the

rabbits, observations on similar lines were made on cats though these were not so fully investigated.

It will be seen from Table XIV. that cats died in the same way as rabbits after tissue implantation. Gas and fluid were present in the peritoneal cavities of these animals.

TABLE XIV.—IMPLANTATION OF MINCED TISSUES INTRAPERITONEALLY.

Cat no.	Tissue inserted.	Dose per kilo, c.c.	Hours before death, at least.
1	Liver.	11	21
2	Muscle.	24	21
4	Liver.	8	Survived.
5	,,	10	17
6	Muscle.	27	17
7	,,	20	17 (killed)

In a series of 4 cats into which autoclaved liver was put, 3 survived and 1 died of pneumonia.

An attempt was made to repeat on cats the observations made on rabbits when a very rigorous aseptic technique was used. Of 3 animals, 2 died with infected liver in their peritoneal cavities while a third survived (Table XV.). It will again be noted that there was considerable hæmoconcentration, but this was present to the same extent in the survivor as in the ones which died. From this small series it appears probable that it is much more difficult to obtain sterile cat than rabbit liver.

TABLE XV.—IMPLANTATION OF MINCED TISSUES INTRAPERITONEALLY.

Cat no.	Tissue inserted, autoclaved.	Dose per kilo, c.c.	Result.
8	Liver.	12·5	Survived.
9	,,	11	,,
10	Muscle.	20	,,
11	,,	18	Pneumonia.

TABLE XVI.—IMPLANTATION OF MINCED TISSUES INTRAPERITONEALLY WITH EXTREME ASEPTIC PRECAUTIONS.

Cat no.	Tissue inserted.	Dose per kilo, c.c.	R.B.C., per cent.	Result.
14	Liver.	17	+29	Died $20\frac{1}{2}$ hrs. Infected.
16	,,	10	+28	Survived.
18	,,	10	+16	Died under 46 hrs. Infected.

Moon [1938] states that doses of pulped liver of 3 to 5 g. per kg. caused the death of 25 guinea-pigs in from 24 hours to several days.

In one small series (Table XVII.) we were unable to confirm this though larger doses were given in some cases.

Table XVII.

Rat no.	Material.	Dose in c.c. per kg.	Result.
1	Chopped liver.	11	Survived.
2	Mashed liver.	5·5	,,
3	,, ,,	5·5	,,
4	,, muscle.	11	,,

Again in a series of 4 rats (Table XVIII.) we did not find that large doses caused death.

Table XVIII.

Guinea-pig no.	Material.	Dose in c.c. per kg.	Result.
1	Mashed liver.	10	Survived.
2	,, ,,	7	,,
3	,, ,,	3	,,
4	,, muscle.	5	,,

We did not therefore pursue the matter further in these animals.

Discussion and Summary.

It is quite clear that in the rabbit and cat it is possible to produce death rapidly by inserting muscle or liver into the peritoneal cavity. In the fatal cases, however, it appears that the liver or muscle is infected with anaerobic gas-producing bacilli. When we succeeded in transplanting liver in a sterile fashion in rabbits, death did not occur. Large doses of sterile peptic digests containing permeability-increasing polypeptides caused death only occasionally unless contaminated, although hæmoconcentration was usually present.

We were not able to confirm Moon's results on guinea-pigs nor did our rats die after liver implantation. It must be emphasised that in comparison with muscle liver is a tissue which autolyses relatively easily, even when sterile. Moreover, in our experiments large amounts of liver were used. In addition, this was minced into a paste to give maximum opportunity for autolysis and subsequent diffusion of the digestive products. Nevertheless from their behaviour the animals did not appear to be "shocked."

rabbits, observations on similar lines were made on cats though these were not so fully investigated.

It will be seen from Table XIV. that cats died in the same way as rabbits after tissue implantation. Gas and fluid were present in the peritoneal cavities of these animals.

TABLE XIV.—IMPLANTATION OF MINCED TISSUES INTRAPERITONEALLY.

Cat no.	Tissue inserted.	Dose per kilo, c.c.	Hours before death, at least.
1	Liver.	11	21
2	Muscle.	24	21
4	Liver.	8	Survived.
5	,,	10	17
6	Muscle.	27	17
7	,,	20	17 (killed)

In a series of 4 cats into which autoclaved liver was put, 3 survived and 1 died of pneumonia.

An attempt was made to repeat on cats the observations made on rabbits when a very rigorous aseptic technique was used. Of 3 animals, 2 died with infected liver in their peritoneal cavities while a third survived (Table XV.). It will again be noted that there was considerable hæmoconcentration, but this was present to the same extent in the survivor as in the ones which died. From this small series it appears probable that it is much more difficult to obtain sterile cat than rabbit liver.

TABLE XV.—IMPLANTATION OF MINCED TISSUES INTRAPERITONEALLY.

Cat no.	Tissue inserted, autoclaved.	Dose per kilo, c.c.	Result.
8	Liver.	12·5	Survived.
9	,,	11	,,
10	Muscle.	20	,,
11	,,	18	Pneumonia.

TABLE XVI.—IMPLANTATION OF MINCED TISSUES INTRAPERITONEALLY WITH EXTREME ASEPTIC PRECAUTIONS.

Cat no.	Tissue inserted.	Dose per kilo, c.c.	R.B.C., per cent.	Result.
14	Liver.	17	+29	Died 20½ hrs. Infected.
16	,,	10	+28	Survived.
18	,,	10	+16	Died under 46 hrs. Infected.

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It may be argued that a rabbit, which is rather a "moist" animal and probably has much body fluid, is not very suitable for experiments of this nature. Nevertheless, on the evidence produced, we believe that those authors who attach great importance to the infection associated with nearly all liver transplantation experiments are right and that autolytic products alone, although producing local loss of fluid, do not produce general effects, after absorption, which lead to a shock-like state.

However, the presence of large masses of autolysing tissue such as muscle must liberate substances of a polypeptide nature which affect profoundly the local blood-vessels, causing increased permeability of the capillaries and hence the escape of plasma. It is possible that this local action of autolytic products may be of considerable importance in the production of wound shock by the promotion of fluid loss. This is a further argument for removing such tissue as thoroughly as possible.

E. P. Abraham and E. Chain are responsible for the chemical work, A. D. Gardner for the bacteriological examinations, and G. M. Brown, H. W. Florey, and A. G. Sanders for the animal work.

We are indebted to the Medical Research Council for a personal grant to E. P. Abraham and E. Chain and a grant towards the expenses of this work.

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ALFRED JOSEPH CLARK, 1885–1941.

OBITUARY NOTICE

ALFRED JOSEPH CLARK, 1885–1941

Alfred Joseph Clark, a member of the Editorial Board of this Journal and a foremost leader in pharmacology, died on 30th July 1941, after an acute illness necessitating surgical operation. His death at the height of his intellectual powers was a great shock to all his friends and colleagues, and it has left those who are concerned with this Journal, in whose affairs he took a vital and intimate part, with an abiding sense of loss. He had been a member of the Editorial Board since Sharpey-Schafer relinquished the editorship and he gave himself unsparingly to the editing of papers, a task for which his fundamental and wide knowledge of biological problems so well fitted him. He was an excellent yet sympathetic critic, and contributors have been quick to recognise the value of his comments on their papers.

Clark was born at Northover, Glastonbury, on 19th August 1885. From Bootham School, York, he went up to King's College, Cambridge, where he took honours in both parts of the Natural Science Tripos. At Cambridge he came under the influence of the late W. E. Dixon and also made contact with the late G. R. Mines with whom he was later to collaborate in an investigation on the action of strophanthin on the excised heart of the frog. He obtained his clinical training at St. Bartholomew's Hospital, and, having qualified M.R.C.S., L.R.C.P., in 1909, and taken the M.B. Cambridge in 1910, he held the posts of house-surgeon at Addenbrooke's and house-physician at Bart's. He took the M.R.C.P. and D.P.H. in 1912 and became successively Demonstrator of Pharmacology at King's College, London, Assistant Pharmacologist at University College, and in 1913 Lecturer on Pharmacology at Guy's Hospital. He took his Cambridge M.D. in 1914, was elected F.R.C.P. in 1921 and F.R.S. in 1931.

On the outbreak of the 1914–18 war Clark took a commission in the R.A.M.C.; he served throughout the war and was awarded the M.C. for gallantry and devotion to duty. In the present war he occupied an important advisory post at G.H.Q. in France, and during the last days of the Battle of Flanders he rendered signal help in the evacuation of the wounded when acting as medical officer in Hazebrouck during the retirement to Dunkirk. He was reticent about his activities

during those strenuous days, but from the little he let drop it was not difficult to realise that many of the wounded owed their ultimate safety to his efforts.

At the close of the 1914–18 war Clark was appointed to the Chair of Pharmacology in Cape Town, but after a year he returned to London to become Professor of Pharmacology at University College, where he succeeded his former chief, the late A. R. Cushny, who had transferred to Edinburgh. Clark's return to University College came at an opportune moment. The pre-war staffs of the Departments of Physiology, Biochemistry, and Pharmacology had dispersed, so that his experience and energy were an invaluable aid to Professor Starling and Sir William Bayliss in their plans for putting the medical sciences once more on a peace-time footing.

In 1926 Clark again succeeded his former chief when he accepted the Chair of Materia Medica in Edinburgh, where he quickly made his personality felt. Students soon realised how much he had their interests at heart and they found in him a staunch friend and adviser to whom they could take their academic problems with the sure knowledge that they would always receive of his best. His *Applied Pharmacology*, now in its seventh edition, illustrates his understanding of the difficulties of the student, for in this book he successfully achieved the integration of pharmacology and therapeutics. In addition, Clark not only interested himself in the athletic activities of the students by sitting on some of their Sports Committees, but found time himself to play Fives or Badminton with the energy and zeal of a man twenty years his junior.

Amidst all his University activities Clark devoted a great deal of time to the development and organisation of the Central Medical Library. The wealth of periodicals and monographs found there at the present time is largely due to the energy he displayed as Convener of the C.M.L. Committee. Research workers in the Faculty of Medicine owe him a great debt for the facilities which this Library affords. It stands as a permanent memorial to Clark's wide outlook and unselfish devotion to the interests of his colleagues, and we are glad to know that this will be recognised and recorded by the placing of a tablet in the Library itself.

It is fortunate that the fruits of Clark's ripe experience in the field of experimental pharmacology have been epitomised and reviewed by his own hand. *The Mode of Action of Drugs on Cells* and *The Comparative Physiology of the Heart*, published in 1923 and 1927 respectively, as well as the volume on general pharmacology in Heffter's *Handbuch der experimentellen Pharmakologie* (1927), reflect the critical nature of his mind, the broad paths he trod, and his extraordinary knowledge of the literature from the earliest times up to the present day. His early training in the fundamentals of the medical sciences was largely

responsible for the successful achievement of such tasks. In all his scientific publications there can be detected the urge to obtain exact and quantitative data on the action of drugs, and it was the natural outcome of his earlier work on the frog's heart that he should use it again to study the metabolism of cardiac muscle. This investigation, in which four of his Edinburgh colleagues collaborated, formed the subject of a monograph, *The Metabolism of the Frog's Heart*, which was published in 1938.

The perfused heart of the frog played an important rôle in Clark's experimental work. In later years he was wont to refer to it as the most valuable biological test-tube at the disposal of physiologists and pharmacologists, and it was indeed remarkable the amount of information he obtained from it in the course of his thirty years' experience. With this small "preparation" he demonstrated how valuable contributions to knowledge can be made by the careful planning of experiments and the use of the simplest apparatus. Those who have worked with him will always remember his dexterity in preparing the heart for closed-circuit perfusion. His hands were not small; yet, after a few deft movements during which the heart was entirely obscured, it finally emerged into view to beat strongly, and continued to do so for hours or even days.

Clark was extremely well read in many subjects and would frequently astonish his colleagues by knowledge which they assumed would be outside the ken of a scientist. He had a playful sense of humour and in the course of friendly discussions would suddenly ask a particularly pertinent question with a charming smile which, to those who knew him well, indicated that he knew the answer better than most. His philosophy was a mixture of realism and idealism which gave him a kindly tolerance towards the shortcomings of his fellow-creatures. He rarely expressed himself strongly on any matter except perhaps on the danger of the uncontrolled trade in secret remedies, ably and courageously exposed in his small monograph on *Patent Medicines* in the "Fact" series.

Clark's services and advice were in great demand by colleagues and institutions alike, and to all he gave freely and willingly. As a member of the Medical Research Council from 1934 to 1938 he did work of great national importance, which was recognised by his re-election, after an interval of one year, to a second term of office. Since his return from France in May 1940 he had worked unceasingly on war problems in his own special field of research. His death in the midst of these labours has not only brought personal grief to many, but is indeed a national loss.

THE INFLUENCE OF SODIUM EVIPAN ON THE HEART AND CIRCULATION. By S. C. DAS. From the Department of Pharmacology, University of Edinburgh.

(*Received for publication 8th May* 1941.)

AMONGST the barbiturates, sodium evipan is very widely used and has gained popularity as an intravenous anæsthetic. Kennedy and Narayana [1935] obtained with evipan a fall of blood-pressure in cats. Bor and Storm [1935] reported that 25 mg./kg. of evipan caused anæsthesia in monkeys, but did not affect cardiac activity and blood-pressure if injected slowly. Tournade and Joltrain [1936] obtained a fall of blood-pressure after evipan and also studied the factors concerned in its production. Stanley-Nowak [1938], from his study of vasomotor and aortic sinus reflexes in dogs, concluded that evipan had "no depressing effect on blood-pressure." In view of the importance of the subject, a detailed study of the effect of evipan on heart and circulation was undertaken to clear these differences of opinion.

Cats under ether and chloralose anæsthesia were mainly used for these experiments; decerebrate and decapitate animals were also used. The intravenous injection of evipan produced a fall of blood-pressure in every case. The extent of the fall varied with the initial pressure: the higher this pressure, the greater being the fall. The effects of a wide range of dosage were measured in one group of experiments. Different animals differed in sensitiveness, but in all cases a sigmoid curve was obtained by plotting the fall of blood-pressure (in mm.) against the logs of the doses (mg./kg.) used (fig. 1). The rapid fall of blood-pressure after a quick i.v. injection was followed by a slow recovery. In cases where fairly large doses were repeated several times, the blood-pressure after recovery failed to reach the original level. Vogt [1930] reported similar experience in her experiments with veronal in cats.

The effects of different rates of injection were next studied. The same quantity produced less fall of blood-pressure if injected in divided doses at short intervals of a few seconds than when given in one dose. A slow continuous injection of more than 1 mg./kg. per minute produced a slow and steadily increasing fall of blood-pressure, but the maximum fall caused by the total quantity given in this way was much less than if given by single or interrupted injections. A continuous

reported diminished vagal activity after evipan, as vagal stimulation produced less slowing of heart's rate after evipan than before it. The author found that vagal stimulation produced less fall of blood-pressure after evipan than before it, thus confirming Tournade and Joltrain's finding. Stanley-Nowak [1934] reported suppression of the carotid sinus reflex by numal, pernocton, and evipan. The carotid sinus reflex was found by the author to be depressed after evipan, but even after a dose of 20 mg./kg. it was not completely suppressed. Tournade and Joltrain [1936] recorded the effects of splanchnic stimulation on kidney volume and observed paresis of peripheral vasomotor apparatus after evipan. The author also obtained similar evidence of weakening of vasomotor activity after evipan by comparing the pressor response obtained before and after evipan, when stimuli of same intensity were given to the sciatic nerve exposed and placed on the shielded electrodes of a secondary coil.

The effect on blood-vessels was determined by perfusion of a leg *in situ* with the animal's own blood. Dixon's pump, as adopted by Robson and Schild [1938], was used with heparin as anticoagulant. The venous outflow was not interfered with. Blood from the central end of the femoral artery was delivered into the perfusion apparatus which rhythmically pumped it onwards into the peripheral end of the artery, thus resembling the natural pumping of the heart, which it substituted with the advantage that it eliminated the depressant action of the drug on the heart that would diminish the peripheral arterial pressure. Carotid pressure was recorded as well as the perfusion pressure. After the perfusion pressure ran steady for some time, 10 mg./kg. of evipan was injected into the femoral vein of the other leg. This caused a fall of the systemic pressure as usual, and also a fall in the perfusion pressure. The rate and force of the pump being constant, a fall in the perfusion pressure indicated vasodilatation. After section of the sciatic and femoral nerves of the perfused leg, the same dose produced less fall of the perfusion pressure. The fall in this latter case was a measure of the direct vasodilatation, whereas the greater fall before section of the nerves represented the combined effects of depression of vasomotor tonus and direct vasodilator effect (fig. 2 (I) and (II)). Direct vasodilatation was also observed on adding evipan to a closed-circuit perfusion, the venous outflow of the perfused leg feeding the perfusion apparatus (fig. 2 (III)). The evidence of this direct vasodilatation effect of evipan is in keeping with Lepper's observation [1924] on the effect of veronal on the perfused isolated rabbit's ear.

Kennedy and Narayana [1935] perfused the isolated frog's heart with evipan and reported that 1 in 4000 of evipan reduced the contractions by 10 per cent., whilst 1 in 1000 caused complete inhibition. Sodium evipan has a higher pH value than frog's Ringer, and unless

infusion avoids a high concentration of the drug caused by a single big injection and hence produces decidedly less depressant effect. An intra-arterial injection also avoids a sudden excess of the drug on the heart, and it was found that when injected into the femoral artery the effect produced was similar to that produced by slow intravenous injection, namely, a slow fall of blood-pressure followed by a still slower recovery. Intra-arterial injections introduce another factor

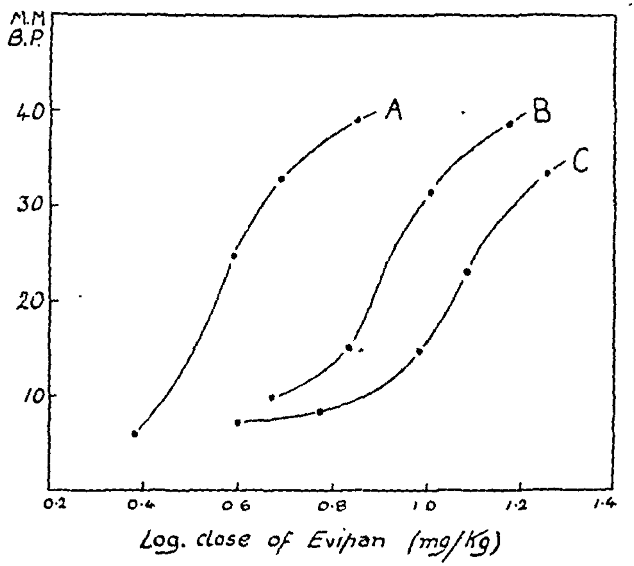

FIG. 1.—Relation between log. dose sodium evipan in mg./kg. (abscissa) and fall of blood-pressure (mm. Hg) in cats (ordinate). A, B, and C are curves obtained in three different animals.

—the probable loss by diffusion or destruction of some of the drug before it reaches the general circulation. The effect of injection into portal vein was also tried and found to be less than when injected into the systemic vein. This was partly due to dilution of the drug in the liver and partly to detoxication in the liver. Results of similar nature were obtained by continuous injection into the portal vein and the aorta.

The next group of experiments were directed towards the analysis of the cause of fall of blood-pressure. That the fall of blood-pressure after evipan was not due to the pH of the solution was shown by the fact that Ringer's solution buffered to the same pH as the evipan solution did not cause a fall, whereas even 2·0 mg./kg. of evipan dissolved in the same quantity of fluid caused an appreciable fall of blood-pressure.

Kennedy and Narayana [1935] stated that they did not observe vagal depression after evipan, but Tournade and Joltrain [1936]

reported diminished vagal activity after evipan, as vagal stimulation produced less slowing of heart's rate after evipan than before it. The author found that vagal stimulation produced less fall of blood-pressure after evipan than before it, thus confirming Tournade and Joltrain's finding. Stanley-Nowak [1934] reported suppression of the carotid sinus reflex by numal, pernocton, and evipan. The carotid sinus reflex was found by the author to be depressed after evipan, but even after a dose of 20 mg./kg. it was not completely suppressed. Tournade and Joltrain [1936] recorded the effects of splanchnic stimulation on kidney volume and observed paresis of peripheral vasomotor apparatus after evipan. The author also obtained similar evidence of weakening of vasomotor activity after evipan by comparing the pressor response obtained before and after evipan, when stimuli of same intensity were given to the sciatic nerve exposed and placed on the shielded electrodes of a secondary coil.

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Kennedy and Narayana [1935] perfused the isolated frog's heart with evipan and reported that 1 in 4000 of evipan reduced the contractions by 10 per cent., whilst 1 in 1000 caused complete inhibition. Sodium evipan has a higher pH value than frog's Ringer, and unless

the evipan solution is buffered to the same pH as frog's Ringer the results cannot be relied on. These authors did not state whether they brought their evipan solution to the correct pH.

The author measured the effects of evipan on frog's heart. The evipan solution was buffered to the same pH as frog's Ringer and each concentration was allowed sufficient time to develop its maximum effect. On the auricle strip of the frog, a concentration of 1 in 100,000 of evipan produced no appreciable change, 1 in 50,000 produced about

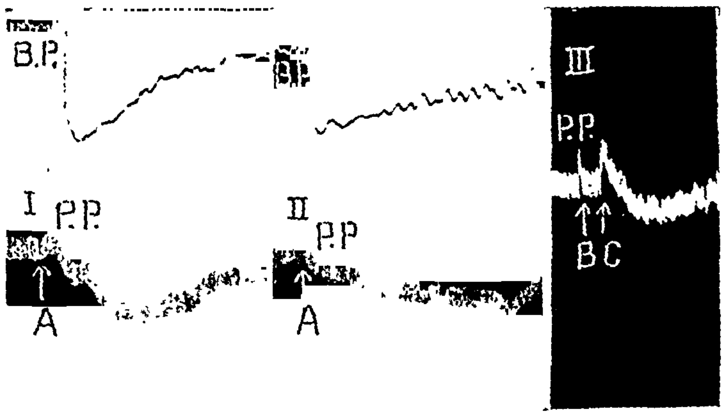

FIG. 2.—Perfusion of femoral artery of cat's leg *in situ*.

BP = blood-pressure and PP = perfusion pressure.

A = injection into femoral vein of sodium evipan (10 mg./kg.).

B and C = injection into perfused artery of 0·5 c.c. saline and 10 mg./kg. sodium evipan in 0·5 c.c. saline respectively.

Time: 1 c.mm. = 90 secs.

I. Sciatic and femoral nerves of perfused leg intact. Vasodilatation produced by injection as indicated by the fall in perfusion pressure.

II. Sciatic and femoral nerves of perfused leg cut. The fall in perfusion pressure is reduced.

III. The evipan injection causes a short initial rise, probably due to the fluid introduced and this is followed by vaso-dilatation.

10 per cent. inhibition, 1 in 20,000 about 20 to 25 per cent., 1 in 10,000 about 40 per cent., 1 in 5000 about 60 per cent., and 1 in 2000 about 95 to 100 per cent. inhibition. Recovery followed washouts if not delayed too long. A range of 25 to 30 times increase in concentration was thus necessary to cause from just appreciable inhibition to complete inhibition. If the percentages of depression are plotted against the logs of the concentrations, an S-shaped curve is obtained (fig. 3).

Tournade and Joltrain [1936] made plethysmographic measurements of the dog's heart. They found that evipan produced dilatation and incomplete contraction, and also obtained evidence of toxic and fatigue effect. The author studied the effects of evipan on the cat's heart, and used the Cushny-myocardiograph to record auricular and

ventricular contractions simultaneously with the carotid pressure. Moderate doses produced marked depressant effects on auricular and

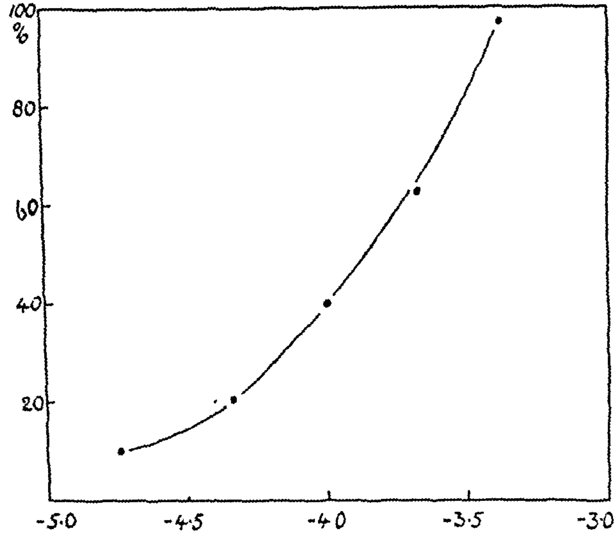

Fig. 3.—Relation between log. concentration of sodium evipan (abscissa) and per cent. depression of contraction of frog's isolated auricle strip (ordinate).

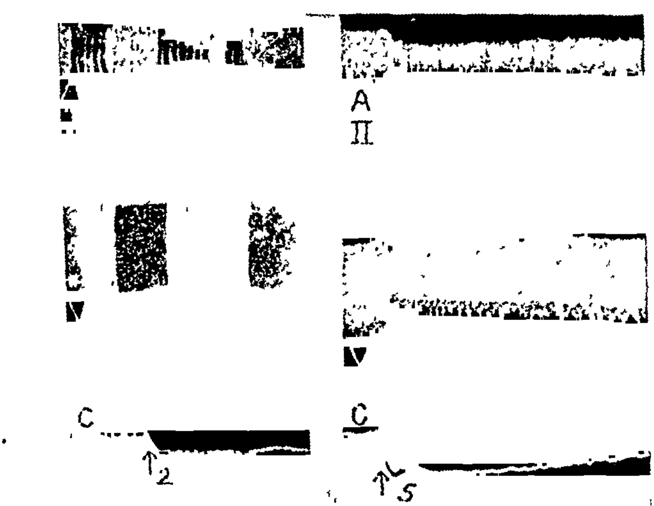

Fig. 4.—Action of intravenous evipan on cat's heart *in situ*. Myocardiographic records of auricle (A) and ventricle (V); blood-pressure (C). Intravenous sodium evipan, 2 mg./kg. at 2, 5 mg./kg. at 5. Tracings I. and II. from different cats. Time: 1 cm. = 2 min.

ventricular contractions. Even a dose of 2 mg./kg. which was only just sufficient to cause an appreciable effect on carotid pressure, produced an appreciable diminution of the auricular contraction (fig. 4).

This shows that the cardiac factor contributes towards the fall of blood-pressure not only in the case of large and toxic doses (as pointed

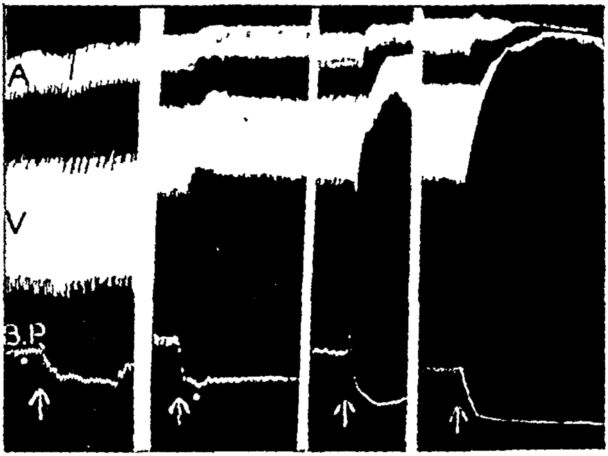

FIG. 5.—Effects on heart and blood-pressure of bleeding and of intravenous evipan.

A and V: Myocardiographic records of auricle and ventricle. B.P.: blood-pressure. Time: 1 cm =2 min.

I. Rapid bleeding of 5 c c./kg. (cat i, 3 kg. B.W.).

II. Rapid bleeding of 5 c.c./kg. (cat ii, 3·5 kg. B.W.).

III. and IV. Intravenous injection of sodium evipan into cat ii. 10 mg./kg. and 40 mg./kg. respectively. The latter injection produced death.

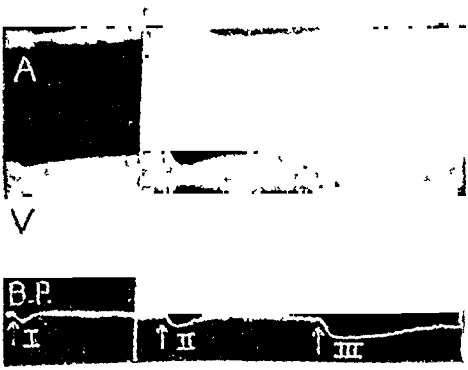

FIG. 6.—Transient nature of action of evipan on cat's heart.

Records as in fig. 5. Time 1 cm =2 min

I., II, and III Injections of sodium evipan 10, 20, and 30 mg /kg respectively. The animal had previously received several doses of sodium evipan

out by Vogt [1930] in the case of veronal), but also in cases of small doses of evipan.

That the diminution of *contraction of the auricle and the ventricle* after evipan was a contributory factor towards the production of fall

of blood-pressure, and was not simply an effect of the fall of blood-pressure brought on by other causes, was proved by showing that a well-defined fall of blood-pressure caused by drawing about 15 c.c. of blood from a femoral artery did not affect auricular or ventricular contraction, whereas a dose of evipan producing a much smaller fall of blood-pressure caused definite depression of auricle and ventricle (fig. 5). The depressant effect of evipan was observed to be more marked on the auricle than on the ventricle. By repeating large doses of evipan several times, during an experiment, complete inhibition of the auricle and extreme depression of the ventricle were obtained, but whereas the ventricle slowly recovered, the auricle paralysis persisted (fig. 6).

Discussion.

Different factors co-operate in the production of the fall of blood-pressure by evipan and other barbiturates. Most workers agree that vasodilatation is an important factor, and some believe vasomotor paresis to be the chief contributory cause. There is also a direct dilator effect on the blood-vessels, as demonstrated by the perfusion experiments. According to Vogt, the cardiac factor comes into play only in cases of severe depression from large doses. It appears that proper consideration has not been given to the part played by the heart in the production of the fall of blood-pressure, probably owing to paucity of accurate quantitative data. Tournade and Joltrain relied on the information given by Kennedy and Narayana, whose method, as already stated, was defective, but the author's experiments show that moderate doses of evipan produce a direct depressant action on the mammal's heart.

The usual human dosage of sodium evipan is 3 c.c. of 10 per cent. solution given quickly, followed by a further 5 c.c. to complete the induction. These quantities correspond respectively to 4 mg./kg. and 10 mg./kg. for an 80 kg. individual. These doses in the cat produce a well-marked depression of auricular and ventricular contractions. If the blood volume of man be taken as 10 per cent. of the body weight, the doses mentioned will produce concentrations in the blood in the heart of at least 1 in 10,000 and 1 in 25,000. These concentrations produce respectively 40 and 15 per cent. depression of the isolated frog's auricle.

The experiments on both the isolated frog's auricle and the intact mammal agree therefore in showing that sodium evipan in therapeutic doses can produce direct cardiac depression.

It is well known that a large dose of evipan rapidly injected intravenously may lead to respiratory arrest. Apart from the risk of respiratory depression, there is also a sudden though short-lived severe circulatory depression, following such rapid administration of evipan

and this probably co-operates with respiratory failure in producing a fatal ending in cases of sudden deaths immediately after a big dose of evipan quickly administered.

Summary.

1. Intravenous injection of sodium evipan always causes a fall of blood-pressure. This fall is not due to the reaction of the solution, but is partly due to vasodilation and partly to cardiac depression.

2. Perfusion of cat's leg *in situ* gives evidence of direct vasodilatation as well as of vasomotor depression.

3. Auricle strip of frog shows 10 per cent. depression with 1 in 50,000 of evipan, 25 per cent. with 1 in 20,000, 40 per cent. with 1 in 10,000, 60 per cent. with 1 in 5000, and 95 to 100 per cent. with 1 in 2000 concentration.

4. Myocardiographic record of cat's heart *in situ* indicates auricular and ventricular depression even with small doses of evipan, showing that the cardiac factor plays a definite part in causing a fall of blood-pressure.

5. This depressant effect on circulatory system can be minimised to a great extent by giving the dose in fractions at short intervals, and still better by continuous intravenous infusion.

The author gratefully acknowledges his indebtedness to Professor A. J. Clark for his advice and help throughout the course of his work.

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THE HUMORAL STIMULATION OF GASTRIC SECRETION.

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The behaviour of the transplanted fundic pouch provided the first indisputable evidence of the participation of humoral agents in the gastric secretory response to a meal [Ivy and Farrell, 1925; Klein and Arnheim, 1932]; and the inhibition of motility and secretion of such a pouch, following a meal of fat, has since been proved to be due to the action of a chalone "Enterogastrone" which has been extracted from the duodenal mucosa [Kosaka and Lim, 1930; Gray, Bradley, and Ivy, 1937]. However, the site of origin and identity of the humoral agent responsible for stimulation of secretion in the transplanted fundic pouch by a meal has not yet been determined. It may originate in either the stomach or the small intestine, and may consist of a hormone or of secretagogues absorbed into the circulation from the gut during the course of digestion. The terms "humoral," "hormonal," and "secretagogues" are to be considered as having the definitions given them by Ivy [1930].

Edkins's [1906] assertion that a hormone "Gastrin," liberated from the pyloric region of the stomach, was concerned in the second or "gastric" phase of the secretory response of the stomach to a meal is now generally recognised to have been based upon inadequate evidence, and has not subsequently received conclusive support, despite a large amount of work on the problem [see Ivy, 1930, for references].

Investigations on the nature of the "intestinal" phase of gastric stimulation led to the suggestion [Kim and Ivy, 1934] that here a humoral mechanism was concerned; but the evidence presented was inconclusive, since in the animal preparations employed some nervous connections remained intact between the stomach and the small intestine.

In order to analyse further the humoral mechanisms involved in the stimulation of a fundic transplant by a meal, it becomes necessary

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to examine separately the effects on such a transplant of the "gastric" and "intestinal" phases of stimulation by a meal.

With this aim in view, the experiments to be described were performed and the following animal preparations made.

Four dogs were provided with a transplanted pouch of the gastric fundus ("transplant") and also a vagotomised pouch of the remainder of the stomach ("main gastric pouch"). Observations were then made of the responses of both pouches to:

(*a*) The feeding of a meal and the oral administration of liver extracts—a potent source of gastric secretagogues [Kim and Ivy, 1934].

(*b*) Application to the mucosa of the main gastric pouch of solutions of secretagogues, histamine, and alcohol for 30–120 minutes ("perfusion" experiments).

(*c*) Mechanical stimulation (distension) of both pouches.

The effect on these responses of anæsthetisation of the mucosa of the pouches with 5 per cent. procaine solutions was also studied.

The results of these experiments have demonstrated that: (*a*) humoral mechanisms are involved in both the gastric and intestinal phases of secretory stimulation by a meal or secretagogues, and (*b*) the gastric humoral agent is probably hormonal in nature.

In order to determine whether or not the presence of the pyloric region of the stomach was essential for liberation of the gastric humoral agent, the main gastric pouch in one of the dogs used in the first series of the experiments was converted into a purely fundic pouch, by the formation from the pyloric region of this pouch of a separate, third, pouch. When the new fundic pouch of this three-pouch preparation was perfused with secretagogues, as in the first series of experiments, a response could still be obtained from both this and the transplanted fundic pouch. Thus participation of the pyloric region is not essential for demonstration of the gastric humoral agent in question.

Surgical Procedures and General Observations.

In three of the four dogs, the transplanted pouches were prepared according to the method of Ivy and Farrell [1925]; in the remaining animal the modification of Klein and Arnheim [1932] was followed.

The most suitable type of dog for the preparation is a large docile female, which has had several litters and possesses large mammæ and loose abdominal skin. At the first operation, a Heidenhain-type pouch of the fundus is placed beneath the skin of the mammary region, being supplied with a single artery and vein selected from the gastro-splenic vessels. In order to conserve the blood supply to the remainder of the stomach, care is taken not to divide the highest branches of the gastro-splenic vessels, which pass to the greater curvature of the

stomach nearer to the cardia, beyond the area which is to be selected for the pouch, and which in most animals form a more or less separate group (figs. 1 and 2).

The second operation is performed about four weeks after the first. A vagotomised pouch of the remainder of the stomach is made, with an end-to-side anastomosis of the œsophagus and duodenum [Ivy,

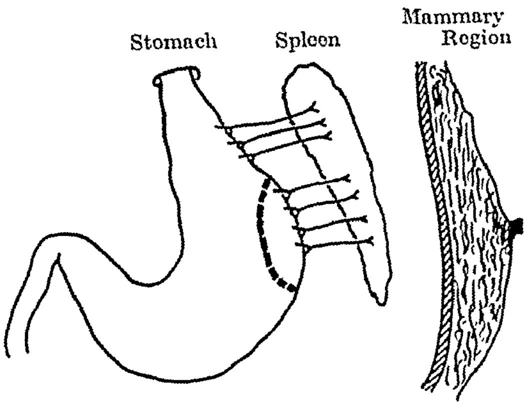

Fig. 1.—Showing the area selected for making the transplanted fundic pouch.

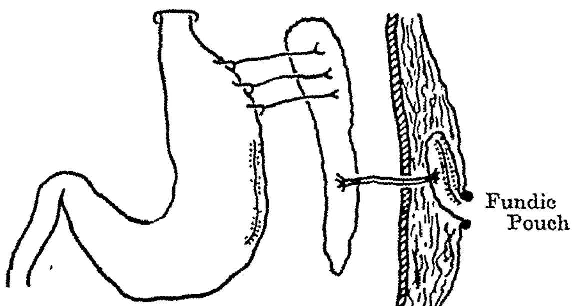

Fig. 2.—The first stage of the two-pouch preparation.

Lim, and McCarthy, 1925]. The pedicle of the transplant is also inspected and its coverings of fat and omentum removed without injuring the vessels, the exposed coats of which are then rubbed gently with gauze moistened in ethyl alcohol. The subsequent fibrosis leads to a gradual diminution in blood supply from this source, causing the transplant to rely to an increasing extent on the new vessels growing in from the surrounding tissues.

The final operation is performed about six weeks or more after the first (above). The pedicle of the transplant is exposed through a small abdominal incision, and the stoma of the pouch watched while the pedicle is clamped with the fingers. If this does not cause

appreciable blanching of the mucosa, the pedicle is divided between ligatures and the abdomen closed (fig. 3). Otherwise, the pedicle is stripped as before and inspected again about four weeks later.

The preparation may also be made in two stages, if it is not desired to make observations of the responses of both pouches before division of the pedicle. The second operation is performed about ten weeks or more after the first, and the transplant pedicle can usually be safely divided on this occasion; if not, it is stripped as described and inspected again a few weeks later.

The four dogs employed in these experiments were maintained in excellent health over a period of several months by following the post-operative treatment and diet employed in this laboratory for dogs completely gastrectomised

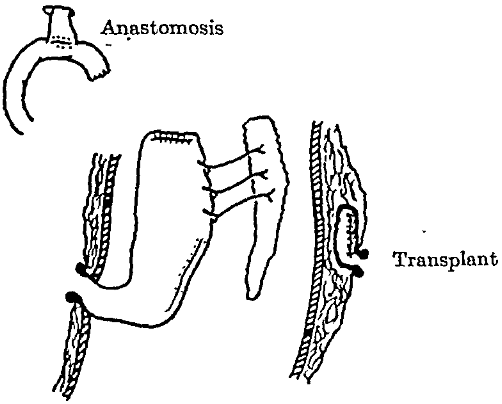

Fig. 3.—The completed two-pouch preparation—fundic transplant, main stomach pouch, and œsophageal/duodenal anastomosis.

or provided with a pouch of the entire stomach. This has been given in detail by Ivy, Morgan, and Farrell [1931] on the basis of several years' experience with such animals.

In two other animals, not otherwise mentioned in this account, the transplant became gangrenous and sloughed a few days after the first stage of the operation, owing to accidental constriction of the pedicle at its exit from the abdominal cavity. This, however, appeared to have no effect on the general health of the animals, which were subsequently used for other experiments.

The mucosa of both the transplant and the main pouch, in the two-pouch preparation, is of normal colour and bleeds readily when gently rubbed with gauze. The best situation for the stoma appears to be at the apex of a mamma; at the first operation a conveniently situated nipple is removed with scissors and the stoma of the transplant brought to the surface of the skin at this point. The resulting elevation of the stoma diminishes the extent of the erosion which often occurs during the second stage, and also facilitates collection of the secretion during an experiment.

The pouches of normal type [Ivy and Farrell, 1925] show spon-

taneous motility when the animal is fasting, and respond by firm contraction to mechanical stimulation, such as massage. The Klein-type pouch [Klein and Arnheim, 1932] has no muscular coat, and is therefore non-motile and incapable of expelling its contents; during an experiment its secretion must be collected by gentle pressure on the skin over the pouch. As Klein noted, the mucosa of the pouch soon becomes perfectly smooth owing to the disappearance of the rugæ, and the stoma shows a tendency to close, necessitating periodical dilatation. Although this pouch is also devoid of Auerbach's plexus, we observed no noteworthy difference in its secretory behaviour, as compared with those of normal type.

Experimental Methods.

The animals were fasted for 12–18 hours prior to an experiment, so that the secretion collected during the preliminary or "control" periods from each pouch was devoid of free acid. This was considered an essential prerequisite in all experiments, in view of the comparatively small responses to be expected, and the necessity therefore of being certain that a production of free acid from either pouch did not represent merely the augmentation of a secretion already proceeding in response to an earlier stimulus, such as the previous meal.

The samples of gastric juice were collected in graduated centrifuge tubes, and were measured and titrated with N/10 NaOH for free and total acidity, using Topfer's reagent and phenolphthalein as indicators. The amounts of acid secreted in each experimental period by the pouches is expressed in the tables as mgs. HCl, free and total. The technique of perfusion of the pouches (*i.e.* the application of solutions to the mucosa) was the same as that employed previously in this laboratory [Kim and Ivy, 1934; Ivy and Whitlow, 1922].

Since commercial liver extracts have been shown to be a potent source of secretagogues for gastric secretion [Kim and Ivy, 1934] they were employed for this purpose in the present perfusion experiments. Amounts of the powdered extracts (Lilly, No. 343, or Wilson), equivalent to 100 g. of fresh liver, were dissolved for use in a volume of water too small to stimulate mechanically by distension of the gastric pouch, *i.e.* 50 c.c. or less.

Experimental Procedure.—The fasting secretion was first collected from both pouches for a control period of 30 minutes or more. If, as was usually the case, this contained no free acid, the main pouch was then perfused at a low pressure (about 5 cm. H_2O) with the secretagogue solution (not more than 50 c.c.) for the required length of time (30–120 minutes). Aliquots of the secretagogue solution were titrated before and after application to the mucosa of the main pouch,

and from the figures so obtained the production of acid in this pouch during the perfusion period was calculated. The subsequent secretion from both pouches was then collected and measured during 30-minute periods, until secretion of free acid from the transplant ceased.

A production of *free* acid by the transplant was regarded as the only reliable criterion of stimulation, owing to the small volumes of juice usually obtained from such pouches, and the comparatively large amounts of mucus associated with low rates of secretion.

1. The Humoral Stimulation of Gastric Secretion from the Stomach and Small Intestine.

A. *Responses to Meals and Oral Administration of Secretagogues.*

When a meal of bread, meat, and milk is fed to a dog with the completed two-pouch preparation, a secretion of free acid is obtained

Table I.

A. *Response of the Two-pouch Preparation to a Meal and the Oral Administration of Liver Extracts.*

Expt.	Dog.	Procedure.	Duration of response (hrs.).	Main pouch. Vol., c.c.	Main pouch. F.A., mg.	Main pouch. T.A., mg.	Transplanted pouch. Vol., c.c.	Transplanted pouch. F.A., mg.	Transplanted pouch. T.A., mg.
136	1	Meal.	4·0	17·2	14·7	31·5	6·8	2·5	6·5
137	1	,,	3·3	20·6	15·0	23·2	6·5	0·8	3·0
141	3	,,	9·5	38·6	30·2	58·5	10·7	6·4	13·0
146 A	4	,,	9·8	14·5	10·4	18·3	3·4	1·4	2·3
138 †	1	Oral administration of liver extract.	6·5	12·8	7·0	15·8	4·0	1·9	3·6
142 *	3	,, ,,	4·3	2·3	0	..	1·7	0	..
143 †	3	,, ,,	7·0	11·8	5·8	13·3	4·5	1·6	3·8

B. *After the Application of Procaine to the Mucosa of the Main Gastric Pouch.*

Expt.	Dog.	Procedure.	Duration of response (hrs.).	Main pouch. Vol., c.c.	Main pouch. F.A., mg.	Main pouch. T.A., mg.	Transplanted pouch. Vol., c.c.	Transplanted pouch. F.A., mg.	Transplanted pouch. T.A., mg.
139	1	Meal.	7·0	18·1	5·8	12·7	4·5	2·3	3·3
144	3	,,	8·0	18·3	30·9	37·0	5·8	3·0	3·9
145	3	,,	10·0	24·4	35·7	43·5	5·2	3·0	4·2
140	1	Oral administration of liver extract.	5·0	9·8	8·6	14·7	3·9	1·1	3·2
146	3	,, ,,	7·5	16·7	24·0	32·6	4·9	2·4	3·3

* Liver extract (8 g. of Lilly No. 343) given in a single dose.
† Liver extract (8 g. of Lilly No. 343) given in four doses of 2 g. each during 1 hour.

from the main gastric pouch and the transplant (Table I., A). This observation proves that the intestinal phase of the gastric stimulation produced by an ordinary meal involves a humoral mechanism. The latent period of the response, and its magnitude, depend mainly on the nature of the meal; raw meat or liver, which passes rapidly through the intestine of these animals, may completely fail to evoke a response from either pouch. This confirms the findings of Ivy, Lim, and McCarthy [1925], who used animals with vagotomised pouches of the entire stomach.

A response of free acid from both pouches is also evoked by the oral administration of liver extracts by stomach-tube, but only when these are given in repeated small doses over an extended period of time of 1 hour or more (Table I., A). Here, as in the case of a normal meal, an important factor is apparently the length of time for which the solution of liver extract remains in the small intestine [Kim and Ivy, 1934].

B. *Responses to Perfusion of the Main Pouch with Secretagogues.*

When the main gastric pouch is perfused for 30 minutes or longer with a solution of secretagogues, a secretion of free acid is obtained from both pouches. Control perfusions of the same duration with a similar volume of warm water or saline have no effect upon either pouch.

These observations (Table II., A) prove that the presence of secretagogues in the stomach causes the entry into the circulation of a humoral stimulant of gastric secretion.

In the animal preparations which we have used in these experiments, the secretion by the transplant regularly appears in 15 minutes after the perfusion of the main gastric pouch has commenced. Prolongation of the perfusion time may increase the response of the transplant to a small extent, but this is usually complete in 1·5 hours from the start of perfusion, whatever the length of time for which the latter is continued (30–120 minutes).

2. The Effect of Mechanical Stimulation of the Gastric Mucosa.

A. *Responses to Distension of the Main Gastric Pouch.*

When the main gastric pouch (containing both fundic and pyloric mucosa) is stimulated mechanically, by distension with a rubber bag or condom filled with 100–200 c.c. of air, and exerting an intra-gastric pressure of 10–15 cm. H_2O, a local response is obtained, as shown by the production of free acid (Table III.).

and from the figures so obtained the production of acid in this pouch during the perfusion period was calculated. The subsequent secretion from both pouches was then collected and measured during 30-minute periods, until secretion of free acid from the transplant ceased.

A production of *free* acid by the transplant was regarded as the only reliable criterion of stimulation, owing to the small volumes of juice usually obtained from such pouches, and the comparatively large amounts of mucus associated with low rates of secretion.

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A. *Responses to Meals and Oral Administration of Secretagogues.*

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Table I.

A. *Response of the Two-pouch Preparation to a Meal and the Oral Administration of Liver Extracts.*

Expt.	Dog.	Procedure.	Duration of response (hrs.).	Main pouch.			Transplanted pouch.		
				Vol., c.c.	F.A., mg.	T.A., mg.	Vol., c.c.	F.A., mg.	T.A., mg.
136	1	Meal.	4·0	17·2	14·7	31·5	6·8	2·5	6·5
137	1	,,	3·3	20·6	15·0	23·2	6·5	0·8	3·0
141	3	,,	9·5	38·6	30·2	58·5	10·7	6·4	13·0
146 A	4	,,	9·8	14·5	10·4	18·3	3·4	1·4	2·3
138 †	1	Oral administration of liver extract.	6·5	12·8	7·0	15·8	4·0	1·9	3·6
142 *	3	,, ,,	4·3	2·3	0	..	1·7	0	..
143 †	3	,, ,,	7·0	11·8	5·8	13·3	4·5	1·6	3·8

B. *After the Application of Procaine to the Mucosa of the Main Gastric Pouch.*

Expt.	Dog.	Procedure.	Duration of response (hrs.).	Main pouch Vol., c.c.	Main pouch F.A., mg.	Main pouch T.A., mg.	Transplanted pouch Vol., c.c.	Transplanted pouch F.A., mg.	Transplanted pouch T.A., mg.
139	1	Meal.	7·0	18·1	5·8	12·7	4·5	2·3	3·3
144	3	,,	8·0	18·3	30·9	37·0	5·8	3·0	3·9
145	3	,,	10·0	24·4	35·7	43·5	5·2	3·0	4·2
140	1	Oral administration of liver extract.	5·0	9·8	8·6	14·7	3·9	1·1	3·2
146	3	,, ,,	7·5	16·7	24·0	32·6	4·9	2·4	3·3

* Liver extract (8 g. of Lilly No. 343) given in a single dose.

† Liver extract (8 g. of Lilly No. 343) given in four doses of 2 g. each during 1 hour.

Table III.

A. *Distension of the Main Gastric Pouch.*

Dog.	Procedure.	No. of Expts.	Duration of response (hrs.).	Main pouch. Vol., c.c.	Main pouch. F.A., mg.	Main pouch. T.A., mg.	Transplanted pouch. Vol., c.c.	Transplanted pouch. F.A., mg.	Transplanted pouch. T.A., mg.
1	Distension of main pouch.	6	2·1	14·1	19·5	37·0	2·4	0	1·1
2	" "	2	2·3	7·7	7·4	11·3	1·3	0	0·5
3	" "	2	2·8	12·6	14·9	18·3	2·2	0	1·2
	Average	(10)	2·4	11·4	13·9	22·2	2·0	0	0·9
	B. *Distension after Application of Procaine to the Main Gastric Pouch.*								
1	Procaine (main pouch).	2	2·0	6·7	0	7·5	1·7	0	1·3
2	" "	2	2·3	3·7	0	3·9	0·9	0	0·7
3	" "	2	2·0	7·0	0	9·9	1·5	0	1·2
	Average	(6)	2·1	5·8	0	7·1	1·4	0	1·1
	C. *Averages of A and B.*								
I. Distension . .		(10)	2·4	11·4	13·9	22·2	2·0	0	0·9
II. Procaine . . .		(6)	2·1	5·8	0	7·1	1·4	0	1·1

The size of the response of the main pouch varies with the pressure employed and its duration; but even when excessive pressures are used (15–30 cm. H_2O), causing signs of nausea in the animal (salivation, restlessness, retching), and are maintained for periods up to 2 hours, *no production of free acid from the transplant is detected,* despite the fact that the amount of free acid secreted meanwhile by the main pouch may be considerably in excess of that evoked by perfusion with liver extracts (Table IV., Expt. No. 152), which has been shown to cause a humoral response from the transplant.

Thus mechanical stimulation of fundic and pyloric mucosa in the main pouch cannot be shown, under the conditions of these experiments, to cause the liberation of a humoral agent of gastric stimulation.

TABLE II.

A. *Perfusion of the Main Gastric Pouch in the Two-pouch Preparation with Secretagogues—Responses of the Main Pouch and the Transplant.*

Dog.	Procedure.	No. of Expts.	Duration of response (hrs.).	Main pouch.			Transplanted pouch.		
				Vol., c.c.	F.A., mg.	T.A., mg.	Vol., c.c.	F.A., mg.	T.A., mg.
1	Perfusion liver extract. 30 minutes, 4·0 g.	11	1·8	14·2	25·4	61·5	2·3	2·8	4·5
2	,, ,,	5	1·8	9·1	1·3	36·1	1·5	0·8	1·2
3	,, ,,	3	2·0	10·7	8·1	33·6	2·6	3·4	4·9
4	,, ,,	3	2·0	3·7	2·1	13·6	1·7	0·9	1·5
	Average	(22)	1·9	9·3	9·2	36·2	2·0	2·0	3·0

B. *After the Application of Procaine to the Mucosa of the Main Pouch.*

Dog.	Procedure.	No. of Expts.	Duration of response (hrs.).	Vol., c.c.	F.A., mg.	T.A., mg.	Vol., c.c.	F.A., mg.	T.A., mg.
1	Procaine to main pouch. Perfusion with liver extract as before.	4	1·5	5·0	0	17·6	1·9	0	0·8
2	,, ,,	3	1·7	2·5	0	9·3	1·0	0	0·4
3	,, ,,	2	1·5	3·4	0	14·6	1·3	0	0·9
4	,, ,,	3	1·5	3·0	0	9·2	0·9	0	0·6
	Average	(12)	1·6	3·5	0	12·7	1·3	0	0·7

C. *After the Application of Procaine to the Mucosa of the Transplanted Pouch.*

Dog.	Procedure.	No. of Expts.	Duration of response (hrs.).	Vol., c.c.	F.A., mg.	T.A., mg.	Vol., c.c.	F.A., mg.	T.A., mg.
1	Procaine to transplant. Perfusion of liver extract as before.	3	1·8	6·0	6·4	20·2	2·2	2·3	3·0
2	,, ,,	3	2·0	4·0	0·5	34·1	1·3	0·4	0·5
3	,, ,,	2	2·0	8·5	6·2	30·7	2·8	3·0	4·4
4	,, ,,	2	1·7	3·6	5·6	13·5	1·1	0·8	1·3
	Average	(10)	1·9	5·5	4·7	24·6	1·9	1·6	2·3

D. *Averages of A, B, C.*

Procedure.	No. of Expts.	Duration of response (hrs.).	Vol., c.c.	F.A., mg.	T.A., mg.	Vol., c.c.	F.A., mg.	T.A., mg.
A. Perfusion of main pouch	(22)	1·9	9·3	9·2	36·2	2·0	2·0	3·0
B. Perfusion after procaine to main pouch mucosa.	(12)	1·6	3·5	0	12·7	1·3	0	0·7
C. Perfusion after procaine to transplant mucosa.	(10)	1·9	5·5	4·7	24·6	1·9	1·6	2·3

3. The Nature of the Gastric Humoral Agent.

A. *The Effect of Procaine on the Humoral Response.*

Savitsch and Zeliony [1911–12, 1913] claimed that the response of the gastric fundus to mechanical or chemical stimulation of a pyloric pouch was abolished by preliminary subcutaneous injection of atropine or anæsthetisation of the pyloric mucosa with 2 per cent. cocaine. They interpreted this as due to the paralysis by these drugs of the efferent and afferent portions respectively of a nervous reflex responsible for the transmission of stimulation from the pyloric pouch to the fundus.

The effects of a local anæsthetic on the stimulation of gastric secretion seem to have been paid little attention subsequently; and since they appeared to offer a means of analysis of the nature of the humoral response of the transplanted pouch, it was decided to extend the work of the above authors, on the two-pouch preparation.

Anæsthetisation of the Main Gastric Pouch.—When the mucosa of the main gastric pouch was anæsthetised by a preliminary perfusion with 50 c.c. of a 5 per cent. solution of procaine for 15–30 minutes (after lavage with warm saline to remove mucus) the subsequent perfusion of this pouch with the usual solution of secretagogues failed completely to evoke a response (production of free acid) from either the main pouch itself or the transplanted pouch. The preliminary perfusion with procaine did not stimulate either pouch (Table II., B).

A 2 per cent. solution of cocaine, as used by Zeliony and Savitsch, was found to have a similar action to procaine, and was employed in the first few trials. However, its use was soon discontinued in favour of procaine, which is less toxic if absorbed into the circulation, and is also largely devoid of vascular effects possessed by cocaine (vasoconstriction) which might have complicated the interpretation of the results.

Weaker solutions of procaine (2 per cent.) were found to diminish but not abolish the humoral response and the local response of the main pouch, on application to the latter. This is consistent with the known relative potencies of cocaine and procaine as local anæsthetics, and also confirms the findings of Lim, Ivy, and McCarthy [1925], who found that a 2 per cent. solution of procaine, when applied to the vagotomised pouch of the entire stomach in a dog, diminished but did not abolish the response to distension.

Anæsthetisation of the Transplant.—When a 5 per cent. procaine solution was applied to the mucosa of the transplant in the same way as to the main gastric pouch, it was found that the transplant still responded to the subsequent perfusion of the main gastric pouch with secretagogues (Table II., C). This shows that procaine does not paralyse the parietal cell.

B. *Mechanical Stimulation of the Transplant.*

In two experiments, balloons were placed in both pouches for the purpose of recording simultaneously changes in motility. That in

TABLE IV.

Showing that Distension of the Main Pouch does not cause the Transplanted Pouch to Secrete.

Only some of the results of the experiments performed are cited here.

Dog.	Expt. No.	Duration of response (hrs.).	Main pouch.			Transplanted pouch.			Remarks.
			Vol., c.c.	F.A., mg.	T.A., mg.	Vol., c.c.	F.A., mg.	T.A., mg.	
1	151	2·5	21·1	36·7	42·8	1·6	0	0·6	Pressure ++, 60 minutes.
1	152	1·8	24·8	57·5	136·0	3·2	0	1·8	,, ,,
3	162	3·0	14·8	17·9	21·7	2·6	0	1·0	,, ,,
3	163	2·5	10·3	11·8	14·8	1·8	0	1·4	,, ,,
2	158	2·5	7·2	10·7	13·1	1·7	0	0·6	,, ,,
Distension of Both Pouches.									
1	153	2·0	16·2	16·5	26·0	1·9	1·0	2·0	
1	154	3·0	9·1	20·1	25·4	4·6	3·6	7·1	
Distension of Transplanted Pouch Alone.									
1	155	2·5	4·6	0	7·8	1·3	0·7	1·4	

the main pouch was distended at intervals to pressures of 15 cm. H_2O or more; and at the conclusion of the experiments a secretion containing free acid was collected from both pouches (Table IV.). This was at first thought to indicate a humoral transmission of mechanical stimulation of the main pouch, since the balloon in the transplanted pouch had been inflated to only a low pressure throughout (5 cm. H_2O). However, when this pouch alone was distended to the same pressure as before, for a similar length of time (about 2 hours), a secretion of free acid was again obtained from it, showing that the response from the transplant in the first instance had been due to local mechanical stimulation. Ivy and Farrell [1925] remark that in one out of several trials they were successful in obtaining a response to mechanical stimulation in their transplants.

entering the circulation during perfusion of the pouch; and (*b*) *a substance of hormonal nature*, liberated from the mucosa of the main gastric pouch by the presence therein of secretagogues.

If the gastric humoral agent consisted of secretagogues absorbed into the circulation during perfusion, procaine might abolish the response of the transplant by preventing the absorption into the circulation of sufficient secretagogues to stimulate the transplant, *i.e.* by decreasing the permeability of the mucosa to secretagogues.

Since there appears to be no way of determining the permeability of the mucosa to such secretagogues directly, an attempt was made to ascertain whether procaine diminished the permeability of the mucosa of the main pouch to other substances known to stimulate gastric secretion when present in the circulation in small concentration, namely, histamine and ethyl alcohol.

The main gastric pouch was first perfused with solutions of: (*a*) histamine, and (*b*) ethyl alcohol, using in each case decreasing amounts until the "threshold" amount of each substance required for stimulation under these conditions was arrived at. Perfusion of these minimal quantities of histamine and alcohol was then repeated after the anæsthetisation of the mucosa of the pouch with 5 per cent. procaine in the usual way. Stimulation of both pouches still resulted (Table V.).

There is no evidence, therefore, from these experiments to show that the permeability of the mucosa of the main gastric pouch to histamine and alcohol is significantly decreased by the previous application of procaine.

Unfortunately, such experiments must necessarily be inconclusive, since it is impossible to prove that procaine does not exert a slight but undetectable effect on the permeability of the mucosa to secretagogues alone, reducing their absorption sufficiently to abolish a humoral response of the transplant.

However, a stronger argument against the view that the humoral agent consists of absorbed secretagogues lies in the fact that although the procaine applied to the mucosa of the main pouch is able there to prevent the secretagogues present during perfusion from stimulating the gastric gland-cells, it is not able, when applied to the mucosa of the transplant, to prevent the gland-cells from responding to the humoral agent which is liberated into the circulation by perfusion of the main gastric pouch with secretagogues. A logical conclusion from this seems to be that *the humoral agent is not the same substance as the secretagogues* which, when perfused in the main pouch, cause the appearance of the humoral agent in the circulation.

If this is accepted, then the humoral agent involved must be of *a hormonal nature*—a substance whose liberation into the circulation from some site in the gastric mucosa is stimulated by the presence of secretagogues in the main gastric pouch. The effect of procaine on

B. *The Effect of Procaine on the Response to Mechanical Stimulation.*

After the preliminary application of a 5 per cent. solution of procaine to the mucosa of the main gastric pouch, mechanical stimulation of this pouch by distension with a balloon at moderate or excessive pressures, as already described, for periods up to 2 hours, always failed to evoke a local response from this pouch, as shown by the absence from the secretion of detectable amounts of free acid (Table III., B).

C. *The Effect of Procaine on the Responses to Meals, etc.*

The application of a 5 per cent. solution of procaine to the mucosa of the main gastric pouch did not prevent the response of this pouch or the transplanted pouch to a meal or secretagogues administered by stomach tube (Table I., B). This observation shows that procaine, whatever the mechanism by which it prevents the liberation of the humoral agent from the main gastric pouch on perfusion of this with secretagogues, is unable to prevent the response of either pouch to the presence in the circulation of a *normal* humoral stimulus, liberated from the intestine by the presence therein of a meal (or of secretagogues).

This is in conformity with the previously observed failure of procaine, applied to the mucosa of the transplant, to prevent the response of this pouch to the humoral agent liberated by perfusion of the main gastric pouch with secretagogues.

4. The Action of Procaine.

The simplest explanation of the results would be that procaine acts by exerting a general toxic effect on the cells of the gastric mucosa, both locally and (after absorption) on the transplant, or that it causes vasoconstriction of the vessels of the mucosa. That neither of these explanations is likely to be the case is clear from the following evidence: (*a*) the application of procaine to the *transplant* does not prevent it from responding to the humoral agent liberated by perfusion of the main pouch with secretagogues; (*b*) the application of procaine to the mucosa of the *main gastric pouch* does not prevent either pouch from responding to the humoral agent liberated elsewhere by a meal or orally administered secretagogues; (*c*) the subcutaneous injection of 25 c.c. of 5 per cent. procaine is found to have no effect on the responses of either pouch to a stimulus.

Two alternatives remain, based respectively on the hypotheses that the humoral agent liberated by perfusion of the main gastric pouch with secretagogues consists of: (*a*) *the same secretagogues,*

the mucosa of this pouch must therefore be to paralyse a mechanism (probably nervous) which is normally responsible for both the local stimulation of the gastric glands by secretagogues and also the liberation into the circulation of the hormonal substance.

It has been shown that there is no evidence from the present work in favour of the liberation of a humoral agent (which would be of a hormonal nature) from the gastric mucosa by mechanical stimulation (distension). However, the application of procaine to the mucosa of the main gastric pouch abolishes the local response to mechanical stimulation, as it does to stimulation by secretagogues. Therefore the mechanisms involved in local mechanical and chemical stimulation, while similar in character, are probably not identical; for the one involves the liberation of a humoral agent, probably of a hormonal character, while the other does not.

Finally the gastric humoral agent, if a hormone, is unlikely to have its site of origin in the parietal cell; for this can apparently be stimulated more strongly by distension than by secretagogues, and yet the former stimulus cannot be shown to liberate the humoral agent.

5. The Pyloric Region as the Site of Origin of the Gastric Humoral Agent.

The pyloric region of the stomach was considered by Edkins [1906] to be the site of origin of the hormone "Gastrin"; and although many attempts have since been made to demonstrate that mechanical or chemical stimulation of this region will evoke a humoral response from the fundus, such experiments have usually given questionable or negative results. There appears to be no conclusive evidence to support this concept of a gastric hormone liberated from the pyloric region of the stomach. However, the idea of a "pyloric hormone" is apparently entertained by some workers even at the present time [Babkin, 1934; Komarov, 1938]; and it has been claimed [Klein, 1935] that after removal of the pyloric antrum from the main portion of the stomach in a dog with a transplanted fundic pouch, this pouch lost its original response to a meal, while retaining its sensitivity to other humoral stimuli (histamine). In view of the results of our experiments with procaine, it was considered of interest to ascertain whether the exclusion of the pyloric region from the main gastric pouch of our two-pouch preparation abolished the humoral response of the transplant to perfusion of the main gastric pouch with secretagogues.

Method.

One of the dogs having a two-pouch preparation was subjected to a further operation, in which the pyloric antrum, together with part

TABLE V.

Showing the Failure of Procaine (5 per cent.) applied to Main Stomach to prevent Stimulation of Secretion of Main Stomach and Transplanted Stomach after the Application of Histamine and Alcohol.

Dog No.	No. of experiments.	Duration of response (hrs.).	Main pouch.			Transplanted pouch.			Procedure.
			Vol., c.c.	F.A., mg.	T.A., mg.	Vol., c.c.	F.A., mg.	T.A., mg.	
			Without Procaine.						
1	1	2·0	11·2	14·1	20·8	2·2	1·3	2·3	25 mg. histamine in 50 c.c. water applied 30 min.
1	1	1·5	12·0	78·6	98·4	2·1	1·3	3·9	,, ,,
1	1	1·5	2·6	35·0	31·7	0·8	0·2	0·3	15 mg. histamine.
1	Ave. 4 expts.	Ave. 1·5	4·0	24·0	39·4	1·2	0·7	0·9	10 mg. ,,
			5 per cent. Procaine applied to Main Stomach.						
1	Ave. 3 expts.	1·8	3·2	28·0	40·9	1·8	0·6	1·3	10 mg. ,,
			Without Procaine.						
2	Ave. 2 expts.	2·25	8·8	66·9	82·1	3·4	7·5	8·6	25 mg. ,,
2	Ave. 3 expts.	1·5	3·6	18·7	26·3	1·5	0·4	0·9	10 mg. ,,
			After Procaine.						
2	1	2·8	11·7	47·4	64·0	5·3	10·9	14·4	25 mg. ,,
2	1	1·5	2·6	19·2	26·3	1·4	0·5	1·2	15 mg. ,,
2	1	1·5	4·4	3·9	11·8	1·2	0·3	0·8	10 mg. ,,
			Without Procaine.						
4	Ave. 2 expts.	2·0	4·1	15·0	24·0	1·5	0·7	1·0	25 mg. ,,
			After Procaine.						
4	Ave. 2 expts.	1·75	2·5	11·5	18·0	1·2	0·2	0·4	25 mg. ,,
			Without Procaine.						
1	1	1·5	20·5	38·0	67·1	6·7	13·0	14·8	7 per cent. alcohol, 50 c.c. for 30 min.
			After Procaine.						
1	1	1·5	11·5	38·8	50·2	4·3	5·5	8·2	,, ,,
			Without Procaine.						
2	1	2·0	6·2	19·1	31·1	1·3	2·6	3·0	,, ,,
			After Procaine.						
2	1	2·0	4·8	23·5	27·3	2·0	1·0	1·4	,, ,,
			Without Procaine.						
4	Ave. 2 expts.	2·0	6·5	20·2	24·1	1·5	1·0	1·3	3 per cent. alcohol, 30 c.c. for 30 min.
4	Ave. 2 expts.	1·75	5·0	17·0	24·0	1·0	0·2	0·6	,, ,,

to the mucosa of the main fundic pouch was found to abolish the response of both pouches to secretagogues; no experiments have yet been made on the response of the procainised transplant to perfusion of the main fundic pouch with secretagogues.

TABLE VI.

A. *Response of the Transplanted Fundic Pouch and the Main Fundic Pouch to Perfusion of the Latter with Secretagogues : Three-pouch Preparation.*

Expt.	Procedure.	Duration of response (hrs.).	Fundic pouch only.			Transplanted pouch.		
			Vol., c.c.	F.A., mg.	T.A., mg.	Vol., c.c.	F.A., mg.	T.A., mg.
166	Perfusion with No. 343 (Lilly) 60 minutes.	2·0	1·9	TR	18·0	1·0	0·3	0·7
167	,, ,, ,,	2·5	2·8	0·5	11·4	1·1	0·3	0·6
168	,, ,, ,,	2·5	1·7	0·2	9·6	1·7	TR	0·9
169	Concentrated liver extract (equivalent to 200 g. fresh liver).*	2·5	2·6	17·2	34·0	1·5	0·5	1·3
B. *After the Application of Procaine to the Mucosa of the Main Fundic Pouch.*								
170	Concentrated liver extract.*	2·5	2·5	0	11·3	1·4	0	1·2
171	,, ,, ,,	2·5	4·4	0	18·4	1·6	0	1·1
172	,, ,, ,,	2·0	1·5	0	1·1	1·4	0	0·8

* Concentrated liver extract was prepared from Lilly No. 343 by precipitation with phosphomolybdic acid and $AgNO_3$ in alkaline solution.

In view of the fact that these experiments have so far been performed only on one dog, they are subject to confirmation. However, the positive nature of the results so far would appear to leave no reasonable doubt that the presence of the pyloric mucosa is not essential for elicitation of the humoral response of the transplanted fundic pouch on perfusion of the main gastric pouch with secretagogues.

CONCLUSIONS AND SUMMARY.

Evidence has been presented that there are at least two components in the humoral stimulation of a transplant of the gastric fundus by a meal—one liberated from the gastric mucosa by the presence of secretagogues, and the other from the mucosa of the small intestine by the presence therein of food or secretagogues. The results of the experiments in which the mucosa of one or other of the pouches of

of the adjoining fundus (to ensure complete resection of the pyloric region), was converted into a third "pyloric" pouch. A pouch was made in preference to removal of this region, as certain perfusion experiments were contemplated at the time of operation; later cir-

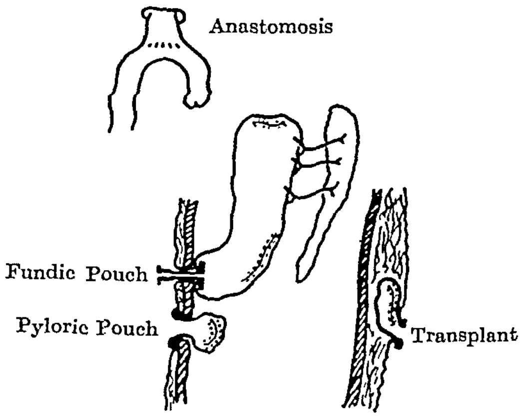

FIG. 4.—The three-pouch preparation—transplant, main fundic pouch, and "pyloric" pouch, with œsophageal/duodenal anastomosis.

cumstances prevented these from being carried out. The remainder of the main gastric pouch (consisting of fundus only) was then closed and a metal cannula inserted (fig. 4). The inclusion of fundic mucosa in the "pyloric" pouch was shown by the occasional presence of free acid in the secretion collected after a meal; it therefore seemed unlikely that any pyloric mucosa could have been left in the fundic portion of the main gastric pouch ("main fundic pouch").

Results.

Since the preparation of the "pyloric" pouch had undoubtedly reduced even further the blood supply to the remainder of the fundus of the main gastric pouch, it was expected that this would no longer respond when perfused with secretagogues. However, in four experiments so far performed, in which the main fundic pouch was perfused with a solution of secretagogues, a response of free acid was obtained from both this pouch and the transplant. The response was smaller than before, owing at least in part to the diminished blood supply to the fundic pouch which remained; but the unmistakable secretion of free acid by both pouches shows that the presence in the main gastric pouch of the pyloric mucosa is not essential for either the local response of the main fundic pouch or the humoral response of the transplant to perfusion of this with a solution of secretagogues. In three experiments (Table VI.) the application of 5 per cent. solution of procaine

ADRENALINE BRONCHOCONSTRICTION IN ISOLATED BLOOD PERFUSED LUNGS.

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(*Received for publication 8th October 1941.*)

IN a preliminary communication, Daly, Mark, and Petrovskaia [1937] reported that in isolated blood perfused dog lungs, single injections of adrenaline following the addition to the circulation of posterior pituitary extracts (Parke-Davis products) produced bronchoconstriction. This was regarded as an abnormal response in view of the fact that with few exceptions [see Golla and Symes, 1913] the vast majority of workers have found that adrenaline relaxes the bronchial smooth muscle. Again, according to experience in this laboratory with perfused lungs of different species, including the dog, guinea-pig, pig, rat, and *Macacus rhesus*, it has been found that injections of adrenaline into fresh lung preparations almost invariably produces bronchodilatation [Foggie, 1937; Daly, 1938; Petrovskaia, 1939 *a*; Hebb, 1940]. Similar results were obtained by Daly, Mark, and Petrovskaia [1937], who found that in three perfusion experiments, injections of adrenaline (5 to 20 μg.) repeated at hourly or longer intervals produced bronchodilatation or had no effect at all on the bronchi up to four hours of perfusion (fig. 1). These were used as control experiments against those in which posterior pituitary extracts were added to the perfusate. Further, in preparations receiving a continuous infusion of an adrenaline solution, they found that superimposed single injections of adrenaline (10–1000 μg.) produced only a weak bronchodilator effect or were without influence on the bronchi (fig. 2). When, however, pituitrin, pitocin, or pitressin was added to the perfusate during the later periods of perfusion (after 90 min. or more), adrenaline injections caused bronchoconstriction. This was observed in eighteen out of twenty-one consecutive experiments. The change in the bronchomotor response was remarkable: the first dose of adrenaline very often increased the tidal air by 10 or 20 p.c., whereas the same size of dose (5 to 20 μg.) given after administration of the hypophysis extracts diminished the tidal air often by as much

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the two-pouch preparation was anæsthetised by procaine have provided indirect evidence that the gastric component of the humoral stimulation is hormonal in nature. No such experiments have been performed in connection with the intestinal phase.

Experiments on one dog only, in which the pyloric region of the gastric mucosa was removed from the main gastric pouch of the two-pouch preparation, lead to the conclusion, subject to confirmation on other animals, that the presence in the main gastric pouch of this region of the stomach is not essential for the demonstration of the humoral response of the transplant to perfusion of the main gastric pouch with a solution of secretagogues.

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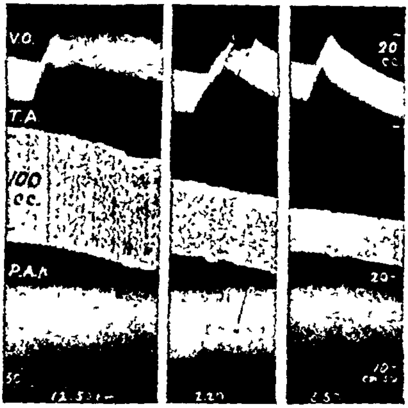


FIG. 1.—Dog, ♂, 22·0 kg. Isolated blood perfused lungs (I.P.L.). Negative pressure ventilation (N.P.V.). Absence of adrenaline bronchoconstriction after 4 hours' perfusion. Perfusion started 11.55 a.m. Adrenaline 10 µg. injected at 12.50, 2.20, and 3.50 p.m. V.O. = blood volume of lungs, an upward movement of the lever denoting a diminution; T.A. = tidal air, downstroke inspiration, upstroke expiration; P.A.p. = pulmonary arterial pressure.

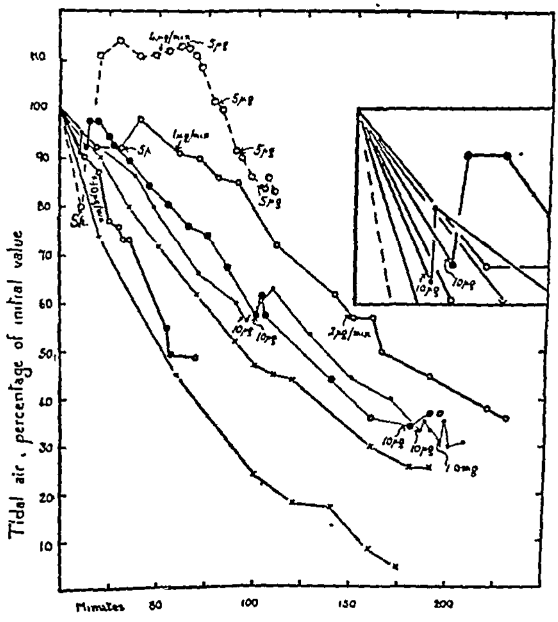


FIG. 2.—Seven experiments showing the gradual diminution in tidal air of lungs perfused with defibrinated blood. Adrenaline infusion or single injections of adrenaline do not alter the rate of tidal air decline but may cause transient bronchodilatation. Inset = enlarged scale of early changes. Sp. = spontaneous change.

as 50 p.c. (fig. 3). Since, in the control experiments in which no pituitary extracts were added, small doses of adrenaline injected at the outset and repeated at hourly or half-hourly intervals caused bronchodilatation or had no effect on the bronchi, it seemed a reasonable assumption that subsequent adrenaline bronchoconstriction was due to the action of the posterior pituitary extracts.

We have now to report, however, that adrenaline bronchoconstriction can be obtained under certain conditions in the perfused lungs of the dog without administration of hypophyseal extracts. When, a few months after publication of the preliminary report by

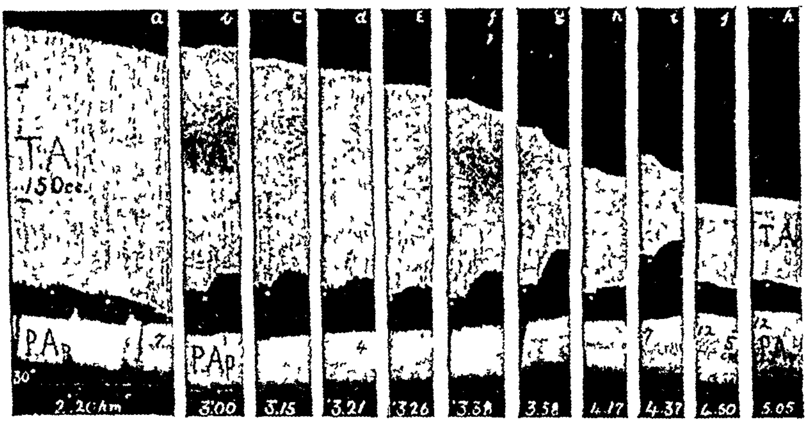


FIG 3—Dog, ♂, 17 0 kg I P L, N P V. Perfusion started 1 50 p m. a = 20 I U. pitressin followed by adrenaline 30 μg, b = 20 I U. pitressin; c, d, e, and f = adr 20 μg; g, h, and i = adr 2, 5, and 5 μg. respectively. Between i and j, ergotoxine ethanesulphonate 10 mg j and k = adr 10 and 20 μg respectively. The numerals on the P A p. tracing denote the mean P.A.p. in cm. of saline.

Daly, Mark, and Petrovskaia, we repeated the control experiments as described above, we discovered that single injections of adrenaline 80–180 minutes after the commencement of perfusion frequently, but not always, caused bronchoconstriction. In general, the bronchoconstriction was not so great as in the experiments in which pituitary extracts had been added to the perfusate, but once it appeared in any given experiment it could be repeatedly demonstrated. This discovery necessitated a re-examination of the whole problem. During this re-examination further experiments were also being conducted on Ringer solution perfused guinea-pig's lungs by Petrovskaia [1939 *b*], who found that adrenaline in some experiments produced slight bronchoconstriction, in others a bronchodilatation. She suggested that the method she employed, which involved recirculation of the perfusion fluid, might lead to retention of tissue substances in the perfusion fluid and be responsible for the abnormal adrenaline response of the bronchial muscle. This opinion was strengthened by the results

of perfusion experiments of other workers in which a continuous replacement of fluid was used: in these, adrenaline nearly always produced bronchodilatation.

On analysis of our experimental records we were unable to find any reason for the difference between the results of the first series of "controls" by Daly, Mark, and Petrovskaia and those performed by ourselves. The experimental procedures in each case were apparently the same, and the suspicion that seasonal variations or the breed or sex of the dogs used had been responsible for the difference was readily eliminated. Moreover, we were unable to account for the absence of adrenaline bronchoconstriction in some but not in other preparations. After careful consideration it was decided that individual variation in the animals used for perfusion was an important factor, and that the problem might be studied to better advantage under improved control conditions, which could be obtained by perfusing separately the right and left lungs of the same animal, the one lung to be used as a control with which the results obtained from the other could be compared. A suitable technique having been devised for this purpose, we commenced a new series of experiments on the separate isolated perfused lung preparation. We desired first to re-examine the results obtained by Daly, Mark, and Petrovskaia—that is, to determine if possible what significance could be attached to the action of hypophyseal extracts in bringing about adrenaline bronchoconstriction; and, secondly, we hoped that by varying the experimental conditions it would be possible to account for the occurrence of this response in preparations not treated with hypophyseal extracts. We propose now to report in detail the results of these experiments.

Methods.

The experiments were all performed on the isolated perfused lungs of dogs. The animals were first bled to death from the femoral artery under local anæsthesia, the blood being defibrinated or heparinised as it was collected and then stored at a temperature of 35–38° C. Next, the sternum was split open and cannulæ were inserted into the trachea, the pulmonary artery and the left auricle. The pulmonary blood-vessels were then washed through slowly with a portion of the stored blood, the lungs being inflated several times during the process. The pulmonary artery having been carefully ligated, all the cannulæ were removed, the heart cut out and the pulmonary veins slit open. The right and left bronchi and pulmonary arterial branches were exposed, and cannulæ inserted into the bronchus and artery of one lung, which was then transferred from the chest to the perfusion apparatus. The other lung was treated in a similar manner. In the perfusion apparatus (fig. 4) the lungs were placed on trays, and perfused

with defibrinated blood at constant inflow by means of Dale and Schuster pumps. The stored blood was usually divided equally between the two circulations.

The method is essentially similar to that described in earlier papers [Berry and Daly, 1931; Alcock, Berry, Daly, and Narayana, 1936], but in the experiments described here the bronchial circulation was not perfused. In connexion with this apparatus there is a further point to be noted: a partition running longitudinally in the respiratory

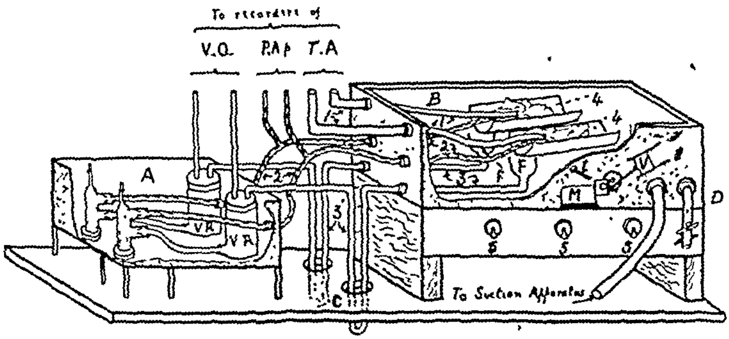


Fig. 4.—Apparatus for perfusing the pulmonary circulations of each lung separately under negative pressure ventilation. A = twin Dale and Schuster pumps driven by separate motors; spiral glass coils (not shown) are fitted inside the water-bath to the output connexions (shaded) of the pumps. B = aluminium respiratory chamber made air-tight with plate-glass cover (not shown). C = U-tubes on venous side of circulations to prevent suction of air into B at each negative pressure cycle. D = adjustable air leak into B. M = electric motor with reduction gear for operating valve (V) for the intermittent admission of air into chamber B. t = thermometer. V.R. = venous reservoirs. 1, 1 = connexions for tidal air (T.A.); 2, 2 = input connexions to pulmonary arterial branches; 3, 3 = venous outflow connexions draining blood from receptacles F, F into venous reservoirs (V.R.); 4, 4 = curved enamel or glass trays containing left and right lungs with cut pulmonary veins from which blood drains into F, F; 5, 5, 5 = switches for heaters controlling temperature of chamber B.

chamber (B) is not shown in the figure. The partition makes it possible to isolate each lung in its own air-tight chamber (when a plate-glass top is put into position and sealed shut with vaseline) and to control the ventilation of each independently of the other. That is, for each chamber there is a separate ventilation system—the suction apparatus (Vactric cleaner), the motor M and valve V being duplicated—by which the negative pressure variations for each chamber can be regulated at will.

In recent experiments we modified these methods in certain particulars. Instead of placing the lungs on trays we suspended each from the bronchial tube cannula which was itself fixed into the wall of the chamber, its one end communicating with the lung and its other end, external to the chamber, connected to the spirometer which recorded the tidal air. Thus the lung was supported entirely by the bronchial ligature. As is well known, there is some disadvantage in

suspending lungs from the bronchus, since the upper lobes are apt to fall over and restrict the blood and air supply. This is especially so of lungs taken from larger animals. The arrangement, however, is more convenient than that shown in fig. 4. Standing beneath the lung was a beaker which acted as the venous reservoir receiving the pulmonary venous blood as it dripped from the opened pulmonary veins. In some of the experiments of this group we used roller pumps [Bayliss and Müller, 1928] instead of Dale and Schuster [1928] pumps. Also in some experiments we set up three perfusion systems consisting of (1) the intact right lung, (2) the two upper left lung lobes, and (3) the lower left lung lobe. In twelve experiments we collected either all or part of the blood in heparin (Jorpes's heparin: 10 mg. per 100 c.c. whole blood) instead of defibrinating it.

Records.—Records taken separately for each lung included tracings of the tidal air (T.A.), pulmonary arterial pressure (P.A.p.), and in some experiments the blood volume of the lung. For measurement of the pulmonary arterial pressure, tambours [Palmer] were used. The changes in lung blood volume were measured by recording the alterations in blood content of the venous reservoir [Daly, 1938].

Graphic Representation of Lung Responses (figs. 5–8, 10).—Our aim was to measure the immediate effect of a drug on the pulmonary arterial pressure, the tidal air and lung blood volume (L.B.V.), as well as any lasting influence which it might have in modifying subsequently the action of some other drug. It became necessary therefore to devise some form of graphic representation of each experiment which would enable us to visualise at a glance not only the general course of the P.A.p., T.A., and L.B.V. changes attributable to the effects of perfusion alone over a period of 2–3 hours, but also the immediate and remote effects of the drug injections. The graphic method employed is briefly described below and is purely arbitrary. The drawing of the graphs has been a somewhat laborious process and, although they do not represent our results with strictly quantitative precision, they have proved invaluable for the purpose of comparing results obtained from a large number of experiments.

In all graphs the L.B.V., T.A., and P.A.p. are arranged from above downwards, the initial values of the last two being taken as 100 p.c. and all subsequent values plotted relative to this figure. The change in L.B.V. is plotted in c.c. The dose of the injected drug is shown in rectangular columns, the responses obtained being shown on the same ordinate. Details of the method of construction will be found in the Appendix. It is sufficient to say here that the mean values of the L.B.V., T.A., and P.A.p. relative to their respective initial values can be seen at a glance at any stage of the experiment, and that the solid black areas intermittently appearing on the L.B.V., T.A., and P.A.p. curves represent diminished lung blood volume, bronchoconstriction,

and pulmonary vasoconstriction respectively, whereas the corresponding unshaded areas represent increased lung blood volume, broncho-dilatation and pulmonary vasodilatation respectively. It will be seen that the lung blood volume gradually increases during perfusion, but that single injections of adrenaline decrease the L.B.V. as indicated by the thickness of the curve (see fig. 5).

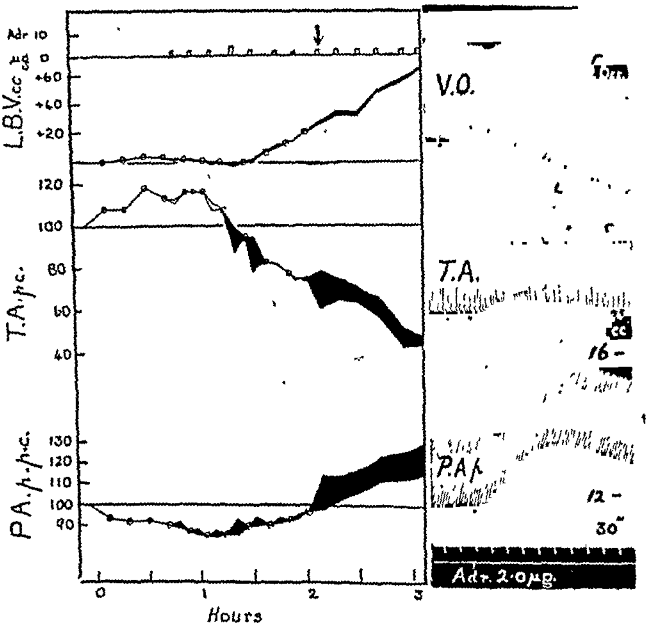


Fig. 5.—Dog, ♂, 8·0 kg. I.P.L., N.P.V. Blood temp. = 37·5° C. Effect of small doses of adrenaline injected at 10-minute intervals. Animal bled to death 10.40 a.m.; perfusion started 11.30 a.m.; 1st injection of adrenaline (2 μg.) at 12.30 p.m. The kymograph tracing shows the response to the 8th injection of adrenaline (2 μg.).

Drugs.—Drugs were injected into the pulmonary arterial tubing or into the venous reservoirs. The following were used: crystalline adrenaline (B.D.H.), or adrenaline solution with 0·5 p.c. chloretone (Parke, Davis & Co.), the two preparations having been found not to differ in activity; ergotoxine ethanesulphonate and atropine sulphate (B.D.H. products); pituitrin, pitocin, and pitressin (Parke, Davis & Co.); and infundin (Burroughs Wellcome Ltd.). We are indebted to Messrs. Parke, Davis & Co. and to Dr. J. M. Robson for highly purified samples of pituitrin, pitocin, and pitressin.

Results.

Spontaneous Appearance of Adrenaline Bronchoconstriction.—Figs. 5 and 6 are typical of the results obtained, in perfusions of the whole lung, from repeated injections of small doses of adrenaline at ten-minute

intervals. One section of the kymograph tracing from which the graph was constructed is shown in fig. 5 for comparison. These experiments and others served as our second series of "controls" and were, as previously mentioned, in direct opposition to the earlier "controls," which showed no adrenaline bronchoconstriction. Adrenaline bronchoconstriction did not occur in every experiment; even so, it seemed remarkable that it had not been previously encountered in the absence of posterior pituitary extract injections. One difference between the

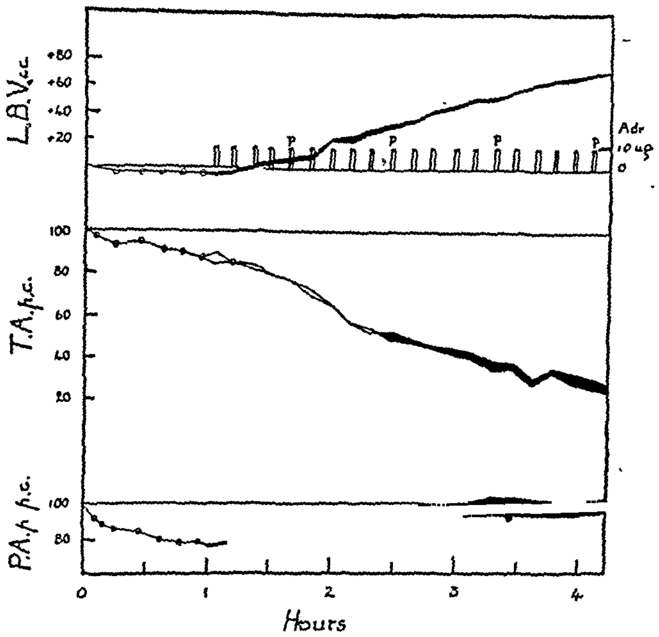


Fig. 6.—Dog, ♀, 11·3 kg. I.P.L., N.P.V. Showing the effect of adr. 10 µg. injected at 10-minute intervals. Adrenaline bronchodilatation is replaced by slight bronchoconstriction. P = adr. + 0·5 p.c. chloretone (Parke, Davis and Co.). Crystalline adrenaline used for other injections.

two series of control experiments was that in the first the interval intervening between the injections was long, whereas in the second it was considerably shorter. In spite of the fact that we had not obtained adrenaline bronchoconstriction in preparations in which adrenaline infusion was carried out, this difference at once suggested that adrenalisation of the blood might itself be responsible for the appearance of the abnormal adrenaline response following single doses. We therefore carried out further adrenaline infusion experiments to test this hypothesis. Adrenaline was infused into the circulation at rates of 2–50 µg./min. and single doses of adrenaline were injected at 10-minute intervals thereafter. On starting the infusion the usual response to adrenaline was obtained, namely, augmentations in the venous outflow

and pulmonary arterial pressure, with or without an increase in tidal air (Table I.). Subsequent single injections of large amounts of adrenaline had no effect on the tidal air in the experiments in which 5 μg. adr./min. or more was being infused. On the other hand, the pulmonary arterial pressure and venous outflow responses generally remained normal although somewhat diminished in size. These results show that the presence of adrenaline infusions greater than 5 μg./min. in the blood diminished the sensitivity of the bronchial musculature to subsequent doses of adrenaline, and to a certain extent diminished the response of the pulmonary vascular bed. No evidence was obtained that previous adrenalisation of the preparations favoured the appearance of adrenaline bronchoconstriction. At this stage of the investigation we turned our attention to separate perfusion of each lung.

TABLE I.—EFFECT OF SINGLE INJECTIONS OF ADRENALINE ON LUNGS PERFUSED WITH ADRENALINISED DEFIBRINATED BLOOD.

No.	Adr. infusion (μg./min.)	Duration (min.).	Effect at start of infusion.			Effect of s.i. adr. during infusion.			S.i. dose (μg.).
			P.A.p.	T.A.	V.O.	P.A.p.	T.A.	V.O.	
1	20	10	+	0	+	+	0	+	10–200
2	5	10	+	+	+	0	0	+	2–25
3	10	33	+	+	+	+	0	+	10
4	50	25	+	0	+	+	0	+	50–500
5	4	67	+	+	0	+	+	+	10
6	5	227	+	+	+	+	0	+	4–10
7	10	..	+	+sl.	0	0	0	..	10–25
8	2	143	sl. +	0	sl. +	+	–	sl. +	10–20

P.A.p. = pulmonary arterial pressure; T.A. = tidal air; V.O. = venous outflow; s.i. adr. = single injections of adrenaline.

Separate Perfusion of Each Lung.—In order to discover how far the physiological conditions of the right and left lung during prolonged perfusion could be regarded as comparable, the pulmonary arterial and bronchomotor responses to single injections of acetylcholine, histamine, and adrenaline into each lung were recorded at intervals for periods of 2–3 hours. The results of one such experiment are graphically illustrated in fig. 7, from which it will be seen that the pulmonary arterial responses of each lung are almost quantitatively identical and the tidal air responses show good agreement. Later experience of over 100 separated lung perfusions showed that the pulmonary arterial responses to adrenaline were closely similar in not less than 95 p.c. of experiments, and the tidal air responses in not less than 85 p.c.

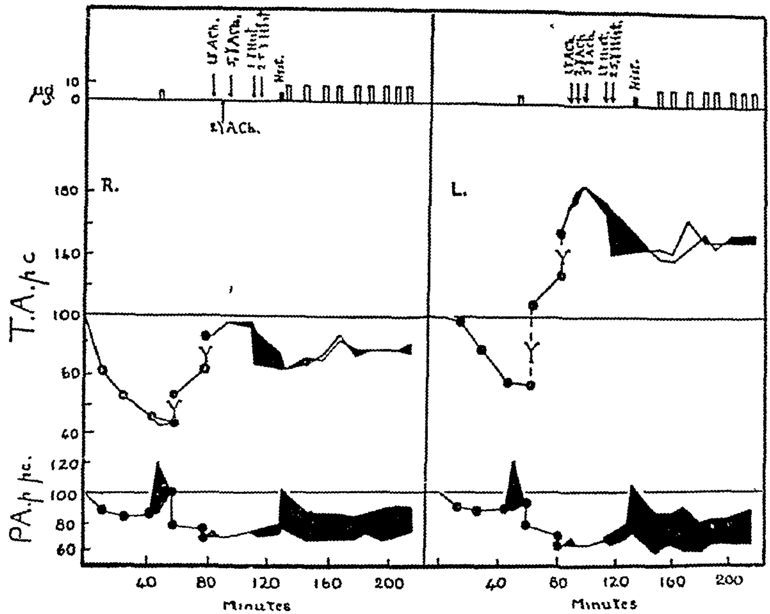


FIG. 7.—Dog, ♂, 13·6 kg. R. and L. lung perfused separately. N.P.V. Injections of adrenaline (unshaded columns), ACh. and histamine were given simultaneously into each circulation. At Y the mean extrapulmonary pressure rhythmically inflating the lungs was lowered.

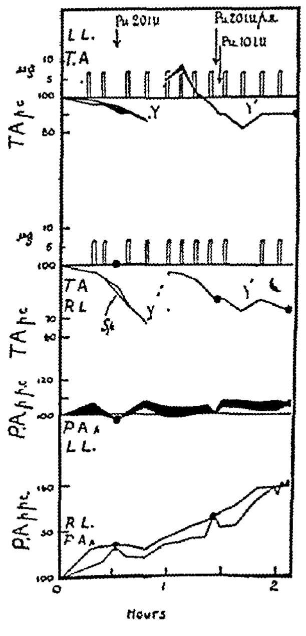


FIG. 8.—Dog, ♂, 19·5 kg. R. and L. lung perfused separately. N.P.V. Vasoconstrictor response to adrenaline (15 μg.) of left lung and vasodilator response to adrenaline (15 μg.) of right lung. Sp. = spontaneous bronchodilatation. Y, Y′ = ventilation pressure changed. Pituitrin (Pu) was added to left lung circulation only.

One experiment is worthy of special mention because it illustrates an extreme variation in the adrenaline response of the pulmonary vascular beds of the two lungs. Repeated doses of adrenaline over a period of 2½ hours produced a fall in pulmonary arterial pressure in one lung and a rise in the other (fig. 8): the bronchi responded poorly to adrenaline. The persistence of a depressor response to repeated doses of adrenaline during 2 hours' perfusion is, in our experience, unique. The pressor response to adrenaline in the left lung of this experiment was not altered by the addition of "pituitrin," a point we had noted in previous experiments. We believe therefore that it would have persisted even if pituitrin had not been added to the blood. Thus the adrenaline depressor response of the right lung, to which no pituitrin had been added, indicates a real difference between the two lungs in respect of their reactivity to adrenaline. This phenomenon can be accounted for by assuming either that adrenaline is not acting upon the same part of the pulmonary vascular bed in each lung, or that the state of the smooth muscle of the blood-vessels in the one lung is different from that in the other. The fact that the animal's own blood was used for perfusion and equally divided between the two lung preparations rules out the possibility of the blood being responsible for the observed difference. Both lungs appeared healthy to the naked eye and both remained in good condition during perfusion. We have one clue which suggests that this difference in response is not due to the site of adrenaline action differing in the two lungs. Rarely, one finds in the perfused lung of the dog that a first dose of adrenaline causes a fall in pulmonary arterial pressure, but subsequent doses, either similar or larger, a rise; accompanying each of these responses is an increase in blood flow from the lungs or no significant change (fig. 9). This result suggests that the action of adrenaline on the vessels governing the blood *outflow* is the same in both cases, but its action on the vessels governing the *inflow* is dilator to the first injection and constrictor to subsequent injections. We are of the opinion, therefore, that the state of the muscle of the pulmonary arterioles is the factor which determined the different arterial pressure responses to adrenaline in the experiment illustrated by fig. 8.

Potential Influence of Pituitrin, Pitocin, and Pitressin in Causing Adrenaline Bronchoconstriction.—Since our later experiments on isolated perfused dog lungs showed that the bronchoconstrictor response to adrenaline might develop without earlier addition of hypophyseal extracts to the circulation, we were led to question whether in the experiments conducted by Daly, Mark, and Petrovskaia the association of adrenaline bronchoconstriction with hypophyseal extract premedication had been merely fortuitous, or whether such premedication, while not actually essential to the response, did potentiate it. To obtain

FIG. 9.—Dog, ♀, 6·6 kg. I.P.L., N.P.V. *a*, 1st injection = saline control; 2nd = 0·5 µg. adrenaline; *b*, 1st = saline control; 2nd = 100 µg. adrenaline. The venous outflow is if anything slightly increased in each case (V.R. tracing). Interval between *a* and *b* = 100 minutes.

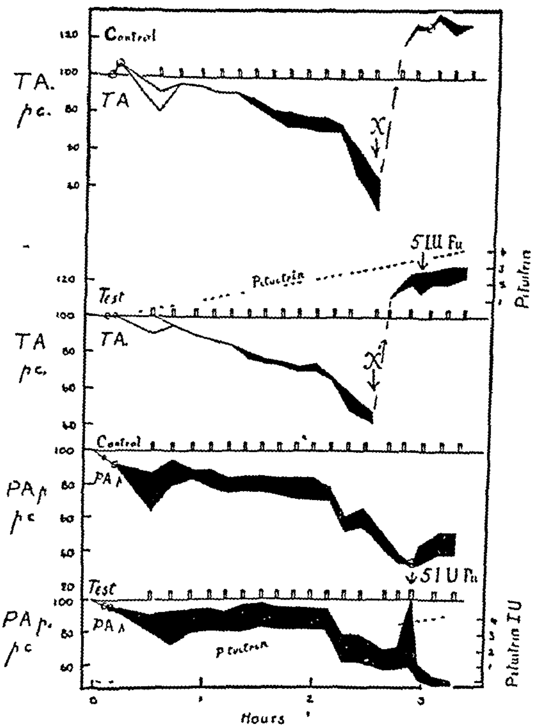


FIG. 10.—Dog, ♂, 29·5 kg. R. and L lung perfused separately. N.P.V. *Control lung*, 5 µg. adrenaline injected at 10-minute intervals. Test lung, infusion of pituitrin 0·02 I.U./min. (dotted line gives total quantity infused) and adrenaline 5 µg. at 10-minute intervals. Pituitrin 5 I.U. was injected at end of experiment into test lung only. xx′ = lungs lightly inflated.

the answer to this question was the immediate purpose of our experiments on the separated isolated perfused lungs.

Retaining one lung as a control, pituitary extracts (pituitrin, pitocin, pitressin, separately or together, and infundin) were added to the circulation of the other, while to both single injections of adrenaline (0·5 μg. and upwards) were given from the outset of perfusion and repeated at ten-minute intervals. The extracts were given by single injection or by steady infusion. The result which was of the most immediate interest was that we were unable to find that any of these extracts either potentiated or otherwise affected the adrenaline bronchoconstrictor response (fig. 10). In certain experiments this response occurred indiscriminately in both the test and the control lung, being sometimes more marked in the one and sometimes in the other. In other experiments both lungs were insensitive or only responded to early injections of adrenaline by bronchodilatation, and adrenaline bronchoconstriction did not develop either with or without the addition of the pituitary drugs. Substantially the same results were obtained in experiments in which we tested the effect of pituitrin administered either at the beginning of perfusion or one or two hours later.

These results served to accentuate that the number of control experiments carried out by Daly, Mark, and Petrovskaia was insufficient to take into account individual variation in the responses of lung preparations. They also focused interest on the more fundamental problem—namely, how to account for the occurrence of adrenaline bronchoconstriction in some lungs when it was absent from others tested under apparently similar conditions. The sporadic occurrence of the response was, in fact, its most puzzling feature, and we realised that further progress in the study of its causes would depend upon our being able to standardise the conditions of the experiments so that we could reproduce the response at will. It is with this aspect of the problem that we were mainly concerned in the subsequent experiments.

Conditions under which Adrenaline Bronchoconstriction Occurs.—In the long series of experiments which followed we set up separated lung perfusion preparations, giving repeated single injections of adrenaline at ten-minute intervals throughout the perfusion period. The general course of the experiments was as follows. In most lungs, adrenaline produced bronchodilatation up to thirty or forty minutes of perfusion, then followed a period of one-half to one hour when no bronchomotor responses were elicited by the injections, after which in certain experiments adrenaline bronchoconstriction would suddenly supervene, while in others, as we had noted earlier, no bronchoconstrictor responses developed. The possible significance of the variation in response was the more strongly impressed on us by the fact that it affected the experiments in groups rather than singly. For example, in an early series, significant adrenaline bronchoconstriction

was regularly observed to develop between 70 and 180 minutes of perfusion (most usually at 120 minutes); whereas in the first experiments of a later series bronchoconstrictor responses either did not occur or were too small to be regarded as significant; while in succeeding experiments of the same series the response again made its appearance.

To account for such discrepancies we thought of two explanations: one, that we were unconsciously altering the technique in some particular way which, though unnoticed at the time, might profoundly affect the subsequent course of the experiment; or two, that there might be pre-experimental conditions, such as dietary or age differences, climatic changes, etc., the effect of which, persisting after the death of the animal, influenced the responses of the perfused lungs. We soon found by control experiments that such variations in technique as may have occurred from time to time were not sufficient to account for the variations in the experimental results. In this connexion we scrutinised each step in the procedure—the collection and preservation of the blood, the wash-through with blood and inflation of the lungs, and the manipulation and dissection involved in their removal—but we could not relate any significant changes in the adrenaline responses of the lung to variations in these procedures.

We also examined the possibility that differences in the temperatures at which the blood and lungs were kept during perfusion might specifically influence the development of adrenaline bronchoconstriction. However, on investigation of a wide range of temperatures—28–40° C. for the blood and 30–50° C. for the air surrounding the lungs—we found no evidence to support this suggestion. In three experiments in which the perfused blood of one lung was kept at a temperature of 37–39° C. and that of the other at 33° C. or less, there were no constant differences in the responses of the two lungs. The only effect on the behaviour of the lungs which could be related to temperature differences was that we found in these and subsequent experiments that with higher perfusion temperatures there often occurred a marked spontaneous and progressive reduction in tidal air, from which there was a certain amount of recovery if the blood temperatures were reduced to 35° C. or less. In fact, we now make it a practice to keep the blood temperatures down to this level throughout the perfusion period; and, although this may seem abnormally low, the result so far has been a definite improvement in the stability of the preparations. It should be mentioned that in all lung perfusion experiments there is always some progressive reduction in the tidal air. In some cases this can be compensated by lightly blowing up the lungs. It may be asked whether there is any relation between the occurrence of this spontaneous reduction in tidal air and adrenaline bronchoconstriction. There did not appear to be any such relation in our experiments, since we have obtained adrenaline bronchoconstriction in preparations with all degrees

of spontaneous tidal air reduction, although it was usual to find, as might be expected, that the superimposed bronchoconstriction was not so marked in lungs which had already a marked reduction in tidal air.

Another line of inquiry concerned the adrenaline preparations used for injection. As stated earlier, two preparations (crystalline adrenaline and adrenaline-chloretone solution) were used for the experiments and were not found to differ in their action. In a number of experiments we observed the appearance of adrenaline bronchoconstriction with either preparation (fig. 6). In further control experiments we also excluded the possibility that the occurrence or non-occurrence of the bronchoconstrictor response depended on *in vitro* changes in the solutions prepared for injection.

We next considered the pre-experimental period. By analysis of earlier results we had found that the occurrence of adrenaline bronchoconstriction did not depend upon seasonal variations or on the sex or breed of the dogs used. Since our experimental animals were maintained under fairly constant conditions, the possibilities of other pre-experimental sources of variation which we could control were limited. In the selection of animals, however, we had not yet considered age as a factor; and with regard to the conditions of maintenance before the experiment we could find at least two ways in which the animals had been subjected to different treatment from time to time, namely, in respect to diet and the temperature of their immediate environment.

As events proved, in none of these factors—age, diet, environmental temperature—did we find the source of the discrepancies in our experimental results. Particular attention was paid in 60 animals to the question of age, because from an earlier series of experiments we had gained the impression that the incidence of adrenaline bronchoconstriction was higher in adult lungs (*i.e.* those taken from dogs of two years or more) than in those of puppies. We found later, however, that the lungs taken from young dogs displayed the same range in respect of their sensitivity to the bronchoconstrictor action of adrenaline as did the lungs of older animals. With regard to the temperature at which the animals were kept prior to being used experimentally, of 35 animals approximately one-third were kept at a temperature of 4–7° C., one-third at 10–15° C., and one-third at 15–20° C. for several days. These extreme variations in environment had no influence on the appearance of adrenaline bronchoconstriction in the lungs subsequently perfused. In the feeding experiments, 25 animals, some of which were on a high protein diet, others on a low, and again others exclusively on carbohydrates, were used for lung perfusions, but the time of onset, incidence, and degree of adrenaline bronchoconstriction were of the same order in all three groups.

The Influence of Time Interval between Adrenaline Injections on the

Onset of Adrenaline Bronchoconstriction.—On reconsideration of the whole problem we were once again struck by the fact that the strongest adrenaline bronchoconstrictor responses occurred after the perfusion had been continued longer than an hour, while in the preliminary period adrenaline produced first bronchodilatation then no effect at all. Now in order to follow the changing response of the lungs we were injecting adrenaline every ten minutes throughout the perfusion period, but we had as yet little information as to how the dosage and the interval of time between single injections of adrenaline during the preliminary period of perfusion affected the later response of the lung

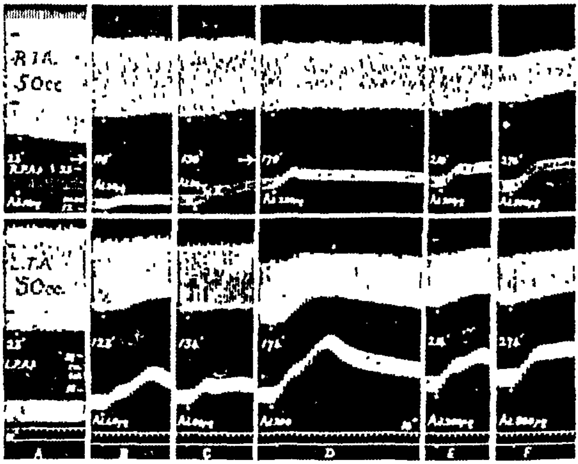


FIG. 11.—Dog, ♀, 18·7 kg. R. and L. lungs perfused separately. N.P.V. *Right lung:* between A and B, 3 injections of adrenaline 20, 20, and 10 μg.; between C and D, 3 injections of adrenaline 50, 200, and 200 μg. *Left lung* no adrenaline injections in intervals between tracings. Adrenaline bronchoconstriction is stronger when injected for the first time after prolonged perfusion.

to the same stimulus; although, as shown earlier, adrenaline infusion rendered the lungs less sensitive to subsequent single injections.

This question was put to the test in some further experiments, in which we took advantage of the separated lung perfusions to compare the effects of varying both the dosage and the time interval elapsing between injections. The results presented in the accompanying table (Table II.) are representative of this whole group of experiments and may be summarised as follows. When no adrenaline was administered before 120 minutes of perfusion an injection at that time always produced bronchoconstriction of a significant order (Table II, Expt A, and fig. 11). Similarly, it was found that within certain limits the less frequent were the adrenaline injections before 120 minutes, the greater was the bronchoconstrictor response at that time. Thus a single injection of adrenaline given during the first 40 minutes of perfusion did not have a sufficiently lasting effect to prevent the occurrence of

TABLE II.—EFFECT OF TIME INTERVAL BETWEEN ADRENALINE INJECTIONS AND BRONCHIAL RESPONSE.

Expt.	Time of perfusion (min.).	Right lung.		Change in T.A. ± p.c.	Left lung.		Change in T.A. ± p.c.
		Injection No.	Dose of adr. (μg.).		Injection No.	Dose of adr. (μg.).	
A	20	1	10	+ 6	..	..	..
	90, 100	2 + 3	10(2 ×)	0	..	..	..
	110	4	10	− 6	..	..	..
	120	5	50	− 6	1	40	− 15
	130	6	50	− 9	2	50	− 7
	140	7	50	− 3	..	..	..
	150	8	200	− 6	..	..	..
	160	9	200	− 3	..	..	..
	170	10	200	− 3	3	200	− 32
B	20, 30	..	..	..	1 + 2	5(2 ×)	+ 3
	40	..	..	..	3	10	+ 3
	50, 60	..	..	..	4 + 5	10(2 ×)	0
	70, 80, 90	..	..	..	6 + 7 + 8	20(3 ×)	0
	100	1	30	− 2	9	40	0
	110	..	..	..	10	40	0
	120	2	50	− 35	11	100	− 5
C	30	1	10	0	1	10	0
	90	2	10	0	2	10	− 2
	120	3	50	− 7	3	50	− 4
	180	4	200	− 21	4	200	− 10
	240	5	200	− 17	5	200	− 11
D	30	1	20	0	1	20	0
	120	2	50	− 35	2	50	− 30
E	30	1	50	+ 7	1	20	+ 1
	120	2	50	− 21	2	50	− 36
F	30	1	50	0	1	10	+ 2
	120	2	50	− 47	2	10	− 45
G	30	1	500	0	1	50	0
	120	2	500	− 78	2	50	− 87
H	30	1	20	+ 9	1	20	+ 8
	120	2	50	− 50	2	50	− 60

T.A. = tidal air; adr. = adrenaline.

moderate adrenaline bronchoconstriction even when the doses were of the order of 500 μg. (see Experiments D to H). Similarly, if the tests were repeated too frequently after 120 minutes the successive bronchoconstrictor responses usually showed a rapid decline, while

with a longer interval between doses (30–60 minutes) good responses might continue to occur for some hours (Experiments A and C). Of particular interest was the finding that if, after a first injection of adrenaline large enough to give an initial blood concentration of 2 μg./c.c., an interval of 90 minutes was allowed to elapse, a second injection gave well-marked bronchoconstriction (Table II., G, H). This effectively disposed of the possibility which we had in mind earlier that a large quantity of adrenaline in the blood prior to death of the animal prevented the appearance of adrenaline bronchoconstriction. At this stage of the investigation we were able to conclude that in freshly perfused lungs adrenaline causes bronchodilatation, whereas in lungs perfused for 60–120 minutes a first dose of adrenaline produces bronchoconstriction; further, that this reversal of adrenaline action fails to appear, or is only moderate, if repeated single injections of adrenaline are given, or if adrenaline is infused into the blood during the earlier periods of perfusion. It also became clear that the interval between injections, rather than the total amount of adrenaline injection, was responsible for the weakness or failure of the appearance of adrenaline bronchoconstriction (Table II.). Along with the differences in sensitivity between one lung preparation and another, it seemed that all our earlier experimental results and those of Daly, Mark, and Petrovskaia could thus be accounted for. Other experiments have confirmed the results in Table II.; in effect the condition had been discovered by which adrenaline bronchoconstriction could be produced at will—namely, prolonged perfusion without the early addition of adrenaline to the blood.

Conditions Governing the Persistence of Adrenaline Bronchoconstriction.—These were examined in a number of separated lung perfusions in which no adrenaline or only a single dose had been given during the first 120 minutes of perfusion, the first injection after 120 minutes causing a well-marked bronchoconstriction. It was found that this response could be best maintained, but not always, by successively increasing the size of the dose (fig. 11, lower tracing). If it were not so increased, the bronchoconstrictor response gradually died away or bronchodilatation occurred. Occasionally when bronchodilatation had so supervened, a larger dose of adrenaline would cause bronchoconstriction once more. The interest of this latter observation is that the condition of the muscle was such that small doses of adrenaline caused dilatation and large doses constriction.

These results differed somewhat from those of our earlier experiments in which repeated single injections of equal strength were given at ten-minute intervals *throughout* the experiment. Weak or moderate bronchoconstrictor responses occurred after 60–120 minutes of perfusion, being initially small but reaching a constant value after 2–6 doses (figs. 5, 6). Thereafter the responses to adrenaline showed variations

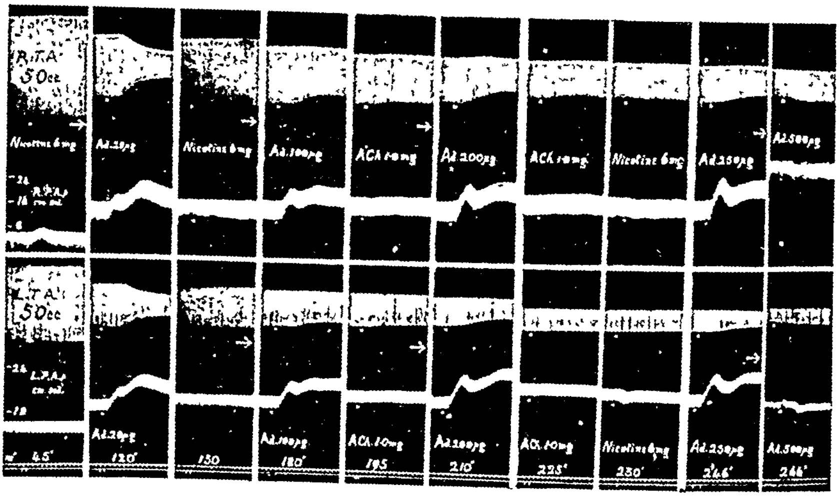
from one preparation to another; in some the bronchoconstrictor response persisted, in others it died away or gave place to a slight bronchodilatation. We interpret these experiments as denoting that one of the conditions governing the persistence of adrenaline bronchoconstriction, once it has become manifest, is the concentration of adrenaline in the blood which existed prior to the appearance of adrenaline bronchoconstriction.

The Effect of Ergotoxine.—Ergotoxine in doses of 1·0–4·0 mg. either suppressed the bronchoconstrictor response to adrenaline or converted it to a bronchodilator response (figs. 3, 12, 13); this confirms earlier work by Daly, Mark, and Petrovskaia [1937]. We also confirmed that ergotoxine suppressed or reversed adrenaline pulmonary vasoconstriction [Daly and Euler, 1932]. These results seem to us to have a special significance in that they can be regarded as evidence that the conditions obtaining in the bronchial muscle when adrenaline constricts it are closely related to those in the smooth muscle of the vascular bed which normally contracts to adrenaline. The acceptance of this view might lead to a dismissal of the possibility that adrenaline in causing bronchoconstriction was stimulating cholinergic nerve endings, but this would be dangerous in view of the fact that ergotoxine may suppress or reverse the pulmonary vasoconstriction and bronchoconstriction produced by acetylcholine [Hebb, 1939, 1940; Petrovskaia, 1939 *a*; Foggie, 1940]. We therefore repeated our tests in atropinised preparations. It was found that bronchoconstriction still occurred in these, and this rules out the possibility that stimulation of cholinergic fibres by adrenaline caused the bronchoconstriction (fig. 12). There still remained one other control—namely, adrenaline tests in nicotinised preparations—in order to eliminate any stimulating action which adrenaline might have upon the intrapulmonary nerve ganglia. This appeared unlikely, but it was thought that prolonged perfusion might make the ganglia sensitive to adrenaline. In the nicotinised preparations, however, adrenaline still caused bronchoconstriction (fig. 12).

The Association of Adrenaline Bronchoconstriction with a Changing Vascular Response to Adrenaline.—In the course of adrenaline tests on isolated perfused lungs we had been struck with the fact that in some preparations the vascular bed exhibited *an increasing sensitivity to* adrenaline as measured by the pulmonary arterial pressure rise. On further examination of this phenomenon, the experiments fell into two main groups, the first in which the onset of adrenaline bronchoconstriction was coincident with an increased vascular response to adrenaline (figs. 5, 6), the second in which no such coincidence could be established; indeed, it was sometimes found that adrenaline bronchoconstriction appeared without the development of any increased vascular sensitivity to adrenaline (fig. 3). When present, the changing vascular response was greater the smaller the amount of adrenaline

injected during the earlier stages of perfusion. In general the maximal potentiation appeared 120 minutes after start of perfusion, or later, and usually amounted to a 100 p.c. increase in pulmonary arterial pressure; in one experiment the increase was 500 p.c.

In the first group of experiments we had to consider whether the apparent potentiation of the vascular response was due to the mechanical effects exerted by coincident bronchoconstriction, for under certain conditions bronchoconstriction causes a passive rise in pulmonary



A B C D E F G H I J

FIG. 12.—Dog, ♀, 16·0 kg. R. and L. lungs perfused separately. N.P.V. *Right lung:* between A and B, nicotine 6 mg.; between C and D, atropine 2·5 mg.; between E and F, atropine 2·5 mg.; between I and J, ergotoxine 1·5 mg. *Left lung:* between C and D, atropine 2·5 mg.; between E and F, atropine 2·5 mg.; between I and J, ergotoxine 2·5 mg. Horizontal arrows indicate interval injections.

arterial pressure in lungs subjected to negative pressure ventilation (unpublished results). The rise in pressure due to this factor is, however, very slight and could only account for a small fraction of the large observed rise. Further evidence against the adrenaline bronchoconstriction and potentiated vascular response being mutually dependent was afforded by a few experiments in which the intensity of either the bronchoconstrictor or the vasoconstrictor response to adrenaline increased while the other diminished. It seemed, therefore, that, while not necessarily interdependent, the two phenomena were related in the sense that they might be regarded as separate manifestations of a more fundamental condition obtaining in the perfused lung preparations. Thus both developed to significant proportions at the same stage of perfusion; both were obscured by too frequent injections of adrenaline; and both were suppressed when the lungs were treated with ergotoxine (0·5 to 1·0 mg.).

The Effect of Added KCl on Adrenaline Bronchoconstriction.—In our search for conditions governing the appearance of adrenaline bronchoconstriction we called to mind some earlier experiments in which we found that the addition of large amounts of potassium to the perfusing blood (sufficient to increase the serum potassium to four times its normal level) at the outset of the experiment was associated with the occurrence of a diphasic response to adrenaline, bronchodilatation followed by marked constriction. In these experiments single doses of adrenaline (2–50 μg.) were injected at ten-minute intervals into both lungs of four separated lung perfusion preparations made from animals bled under novocaine, after KCl had been infused into one or both lung circulations at the rate of 24·0, 27·0, 13·5, and 27·7 mg./min. for 12, 13, 66, and 25 minutes respectively. The other lung, if not infused with KCl, was used as a control or for testing the effect on the adrenaline response of the presence of other substances. There was approximately 500 c.c. of blood in each venous reservoir and 30 c.c. in each lung, so that, neglecting any K retention by the lung tissues, the final concentration of added KCl in the blood of each lung circulation was approximately 54, 66, 168, and 131 mg./100 c.c. or 29, 35, 88, and 70 mg./100 c.c. blood respectively. Dr. A. Brown, working in this laboratory, has estimated the K content of dog's serum (from defibrinated blood). In four experiments he obtained values of 25·8–28·5 mg./100 c.c. The final concentration of serum K in our experiments would therefore be from 2·8 to 6·5 times normal if no potassium entered the erythrocytes.[1]

In all experiments the infusion of KCl caused a progressive diminution in tidal air. It was found that the addition of K to produce 2·8–3·1 times the normal concentration in the serum renders the bronchial muscle more responsive to adrenaline as regards both contraction and relaxation (fig. 13), whereas a concentration of serum K higher than this value renders the muscle less sensitive to adrenaline.

Further points of interest in these experiments require mention. Adrenaline bronchoconstriction and its reversal by ergotoxine to a normal response occurred in preparations to which cyanide (600 μg. NaCN/100 c.c. circulating blood) had been added. Also of interest is the fact that adrenaline bronchodilatation in the KCl infused lung preceded the bronchoconstriction at a time when pure adrenaline bronchoconstriction occurred in the other lung, indicating that potassium also favours the dilator action of adrenaline (fig. 13, *d*). That this action is not dependent upon the contracted state of the bronchi has been shown by the persistence of the effect after the bronchial muscles have been stretched by blowing up the lungs. Again the very early adrenaline bronchoconstriction following a preliminary dilatation in the KCl perfused lung shows that excess of potassium in the blood also

[1] For these calculations the serum is taken as 60 p.c. of the whole blood.

enhances the adrenaline bronchoconstrictor effect. In another experiment in which adrenaline (20 μg.) had ceased to have any effect on the bronchi, the infusion of KCl led to an adrenaline bronchodilatation followed by constriction: we have also seen after 140 minutes' perfusion a potentiation of adrenaline bronchodilatation by KCl infusion.

These experiments suggested to us that the reversal of adrenaline action on the bronchial smooth muscle might have been associated

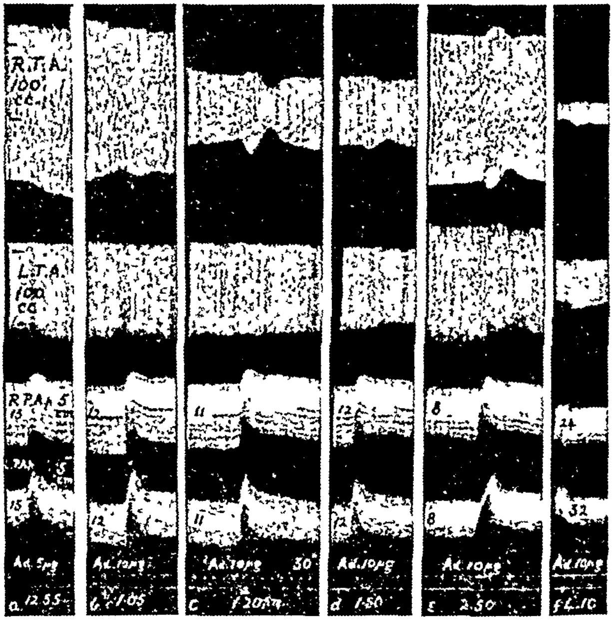


FIG. 13.—Dog, ♂, 33·0 kg. R. and L. lungs perfused separately. N.P.V. *Right lung:* between 12.58 and 1.10 p.m., KCl 24 mg./min. infused into the circulation. *Left lung:* between *b* and *c*, NaCN 150 μg./100 c.c. circulating blood added to venous reservoir; between *e* and *f*, NaCN 300 μg./100 c.c. blood added.

Both lungs were lightly inflated between *d* and *e*, and ergotoxine 2·5 mg. was injected into each lung between *e* and *f*.

with the liberation of extra potassium in the circulating blood, and this possibly because of associated destruction of red blood cells or possibly damage to the lung itself. We have no direct evidence that such is the case, although on making hæmatocrit tests from time to time during the course of some experiments (6 expts.) we found that in these there was always extensive hæmolysis by the end of the first hour and a half of perfusion, if not earlier. Incidentally we found that hæmolysis of the blood during its collection from the opened femoral artery is much less if it is not defibrinated but added to heparin (10 mg. per 100 c.c. blood); but even heparinised blood was hæmolysed to some extent during perfusion. In this connexion it was observed that the Dale-Schuster pump caused somewhat less hæmolysis than

did the Bayliss–Müller roller pump. The adrenaline bronchoconstrictor response, it should be added, was demonstrated both with heparinised and defibrinated blood as the perfusate, and in both cases it occurred at the end of the second hour of perfusion.

Effect of Pituitrin, Pitocin, and Pitressin (Parke, Davis and Co.) on the Lungs.—In the course of our experiments the effects of injecting posterior pituitary lobe extracts were measured. The results of 41, 26, and 32 injections of pituitrin, pitocin, and pitressin (Parke, Davis & Co.) respectively are shown in Table III. With all three drugs

TABLE III.—EFFECT OF PITUITRIN, PITOCIN, AND PITRESSIN ON ISOLATED PERFUSED LUNGS OF THE DOG.

	Dose I.U.	P.A.p.	T.A.	V.O.	No. of obs.
Pituitrin	5	– sl.	–	+	2
	10	– sl.	0	0	1
	10	– sl.	–	n.t.	1
	3–10	+	–	+	33
	10	+	–	n.t.	3
	10	0	–	+	1
	O.U.				
Pitocin	10	– sl.	–, +	+	1
	3	0	–	0	1
	5–10	0	–	+	2
	5–10	+	–	+	8
	5	+	–	n.t.	3
	5–20	+	0	+	9
	3	0	–	0	1
	10	0	0	+	1
	P.U.				
Pitressin	5	– sl.	–	n.t.	1
	5	– sl.	+	–	1
	5	+	–	n.t.	1
	10	+	0	+	1
	5	+, –	–	n.t.	1
	5–20	+	–	+	27

P.A.p. = pulmonary arterial pressure; T.A. = tidal air; V.O. = venous outflow; I.U. = international units; O.U. = oxytocic units; P.U. = pressor units; n.t. = not taken.

the most usual effect was an increase of P.A.p. and venous outflow and a bronchoconstriction. It was noticed, however, that on the rare occasions in which a slight decrease of P.A.p. occurred it was always following the first injection of the drug—subsequent injections producing an increase of P.A.p. This phenomenon recalls the observations which have been made in this laboratory [Daly, 1938; Petrovskaia, 1939 *b*; Daly, Foggie, and Hebb, 1940] concerning the somewhat rare adrenaline

enhances the adrenaline bronchoconstrictor effect. In another experiment in which adrenaline (20 μg.) had ceased to have any effect on the bronchi, the infusion of KCl led to an adrenaline bronchodilatation followed by constriction: we have also seen after 140 minutes' perfusion a potentiation of adrenaline bronchodilatation by KCl infusion.

These experiments suggested to us that the reversal of adrenaline action on the bronchial smooth muscle might have been associated

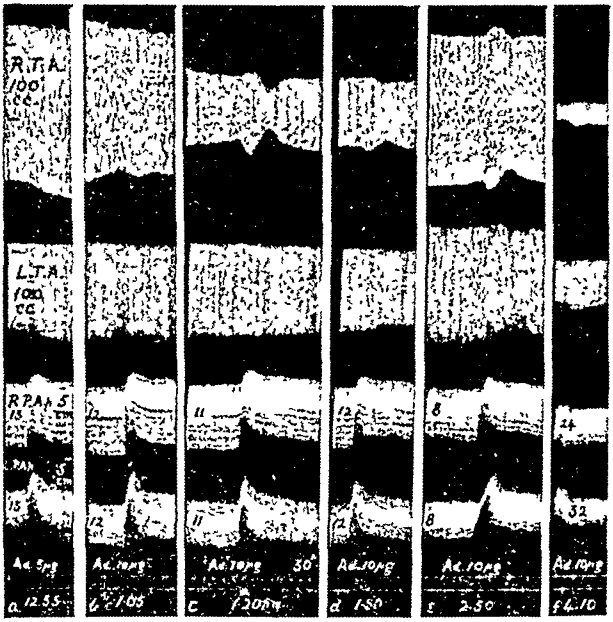


FIG. 13.—Dog, ♂, 33·0 kg. R. and L. lungs perfused separately. N.P.V. *Right lung:* between 12.58 and 1.10 p.m., KCl 24 mg./min. infused into the circulation. *Left lung:* between *b* and *c*, NaCN 150 μg./100 c.c. circulating blood added to venous reservoir; between *e* and *f*, NaCN 300 μg./100 c.c. blood added.

Both lungs were lightly inflated between *d* and *e*, and ergotoxine 2·5 mg. was injected into each lung between *e* and *f*.

with the liberation of extra potassium in the circulating blood, and this possibly because of associated destruction of red blood cells or possibly damage to the lung itself. We have no direct evidence that such is the case, although on making hæmatocrit tests from time to time during the course of some experiments (6 expts.) we found that in these there was always extensive hæmolysis by the end of the first hour and a half of perfusion, if not earlier. Incidentally we found that hæmolysis of the blood during its collection from the opened femoral artery is much less if it is not defibrinated but added to heparin (10 mg. per 100 c.c. blood); but even heparinised blood was hæmolysed to some extent during perfusion. In this connexion it was observed that the Dale-Schuster pump caused somewhat less hæmolysis than

blood perfusing the lungs prevents the appearance of adrenaline bronchoconstriction, it is not improbable that the presence of adrenolytic substances in the blood and the adrenaline content of the blood prior to and during bleeding influence the ultimate responses of the perfused lungs by determining the "adrenaline background" of the bronchial smooth muscle. It has already been mentioned that an increased vasoconstrictor response to single injections of adrenaline is most easily demonstrated in preparations receiving only small amounts of adrenaline during the earlier stages of perfusion. This result, taken in conjunction with our observations on the conditions governing the appearance of adrenaline bronchoconstriction, suggests that the absence of an adrenaline "background" increases the propensity of the smooth muscle of the lungs as a whole to respond by contraction to single injections of adrenaline—whether the more usual response to the latter is relaxation or contraction. Thus a condition of the bronchial and vascular musculature arises in which their responses to a sudden presentation of adrenaline tends to deplete the lungs of blood and air. No two co-existing responses of the lungs more disadvantageous to oxygenation of the blood can be imagined.

Without further information it would be idle to speculate in any detail on the significance of our results in respect of adrenaline action on smooth muscle in general. We have, however, a clear demonstration that certain artificial conditions reverse the normal action of adrenaline on a smooth-muscle type which has usually been recognised as remarkably constant in its response to adrenaline. Whether the mechanisms responsible for this change are determined by abnormal enzyme activity, the failure of normal persisting nervous influences, or the absence of normal humoral conditions in the blood, must be left to future experimentation. It may be that none of these will eventually prove to be the significant factor, yet there is one outlook which appeals to us in directing attention to the lines on which future investigation should be conducted. We refer to the implication of our results from the clinical point of view.

The interesting action of adrenaline in causing bronchoconstriction under specified conditions appears to us of sufficient importance to warrant speculation as to the occurrence of an analogous effect in the human body. We have in mind the part which adrenaline may play in promoting or cutting short attacks of paroxysmal dyspnœa due to bronchospasm in human subjects with a neuropathic and, according to some authorities, of necessity an allergic diathesis. It has always appeared remarkable to us that an emotional stimulus which would be expected to be accompanied by an increased secretion of adrenaline should in some cases of asthma promote and in others stop (just as adrenaline injections do) an attack of paroxysmal dyspnœa. This curious paradox, well recognised in clinical practice, has been responsible

fall of P.A.p. in freshly perfused lungs of the dog, monkey, and pig (although more common in the pig), and the adrenaline rise of pressure which takes place as a result of later injections. We have also found that the rise of P.A.p. due to injections of pitressin may become larger with each successive injection of the drug.

Discussion.

Our results demonstrate that when isolated lungs are perfused with blood for prolonged periods the bronchomotor and vasomotor responses to adrenaline show wide variations from one animal preparation to another, whereas the responses of freshly perfused lungs exhibit a remarkable constancy. It has also been shown that the adrenaline responses of each lung (taken from the same animal) when separately perfused and ventilated are comparable with one another in the vast majority of experiments, even in prolonged perfusions, although on rare occasions marked differences, both quantitative and qualitative, have been observed. It follows that a perfused lung preparation taken from one animal cannot be safely used as a control against one taken from another animal except during the early stages of perfusion, and the method of choice for obtaining control and test preparations is to perfuse the right and left lung separately. In the experiments of Daly, Mark, and Petrovskaia, in which the appearance of adrenaline bronchoconstriction after prolonged perfusion was attributed to the presence of added posterior lobe extracts, the control experiments consisted of prolonged perfusion of lungs taken from other animals, and here adrenaline bronchoconstriction did not appear. They were unaware that adrenaline bronchoconstriction occurs spontaneously in some preparations during prolonged perfusion but not in others, and the fact that it appeared in the preparations to which the extracts were added and not in the others appears to have been entirely fortuitous.

The conditions which favour the appearance of adrenaline bronchoconstriction are prolonged perfusion and the absence of adrenaline additions to the blood during the first 60–120 minutes of perfusion; nevertheless the magnitude of the abnormal response varies greatly in different lung preparations. This, in theory, might have been due to the previous history of the animal, the condition of the blood perfusate, and the skill with which the preparation was set up. Deliberate alterations in the last two factors, however, did not appear to have a great influence either on the incidence or magnitude of adrenaline bronchoconstriction. This result suggests that the condition of the animal prior to being bled under local anæsthesia or its physiological reactions during the process of bleeding, which may also determine the physiological properties of the shed blood, in part govern the responses of the isolated lungs. Since adrenaline added to the

Regarding the rôle of the adrenal glands, this hypothesis carries with it the assumption that partially exhausted glands are still capable of secretion in response to a strong emotional stimulus, or that the extra adrenaline secreted during emotion escapes destruction in the blood in an amount sufficient to reach the lungs and act upon the bronchi. In this connexion we wish to point out that the opinion has often been expressed that attacks of asthma are associated with periods during which signs of adrenal exhaustion are in evidence.

With regard to the general experience that injections of adrenaline or emotion may cut short an attack of asthma, some of our observations on perfused lungs may throw light on the phenomenon. It will be remembered that in some of our experiments repeated single injections of adrenaline of the same order or smaller than those causing bronchoconstriction, when given subsequent to the appearance of adrenaline bronchoconstriction, tend to be without effect or to cause some relaxation of the bronchial muscle. This phenomenon may also have its counterpart in the effect of slowly absorbed subcutaneous injections of adrenaline, or of a second emotional release of adrenaline, in human subjects in relieving a bronchospasm which has been initially brought on by a *sudden* outpouring of adrenaline from the adrenal glands (or adrenergic fibres to the bronchi) acting upon an already adrenaline-starved bronchial muscle. However this may be, these views serve to stress that the main value we place upon our results rests upon the recognition that adrenaline may under certain conditions constrict or dilate the bronchi according to the dose, and depending upon the concentration of adrenaline to which the bronchi have been previously submitted. If our speculations have any foundation in fact, we are able to visualise in a subject with an asthmatic diathesis accompanied by hypoadrenalinæmia, an asthmatic attack brought on by an emotional release of adrenaline from the adrenal glands or bronchial muscle adrenergic fibres, and subsequently relieved by a second emotional release of adrenaline.

The fact that adrenaline injections, however large the dose, readily dilate bronchi which have been previously constricted by drugs such as histamine or pilocarpine, emphasises that the conditions under which adrenaline is acting in these circumstances are entirely different from those in our experiments, and heighten rather than lessen the stress which we wish to lay upon the condition of the muscle in determining its response to adrenaline.

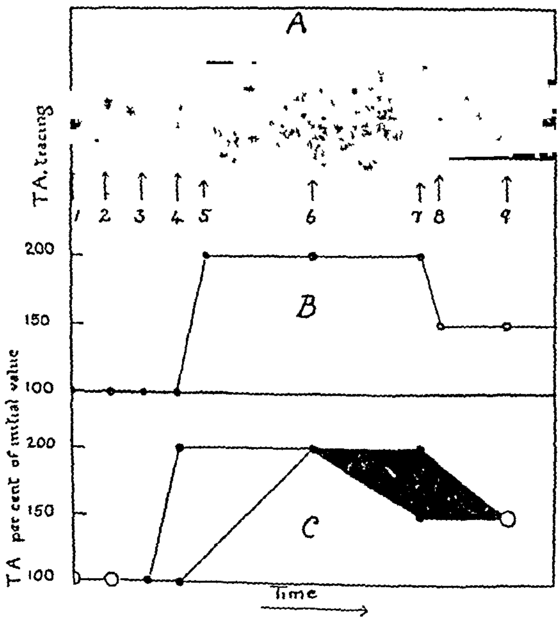
We recognise that the parallel between the results of our experiments on the one hand and certain clinical observations on the other may be entirely fortuitous, the more so because we have selected certain clinical observations and attempted to interpret them in the light of our own work. It is also true that other phenomena associated with asthmatic attacks have been given no place in this comparison, which,

in no small measure for our continued interest in the action of adrenaline on the lungs. This interest has been increased by the fact that atropine, even in large doses, rarely results in the termination of an attack of asthma. Taking all the evidence together, it seemed reasonable in such cases to implicate the sympathetic rather than the parasympathetic nervous system, and to speculate whether adrenaline, released from the suprarenal glands, or at the termination of adrenergic fibres supplying the bronchial muscle, might, when promoting or alleviating an attack of asthma, be acting under entirely different local conditions. This suggestion is in no way intended to minimise the importance of the evidence that in some cases of bronchospasm the underlying mechanism involves stimulation of vagal bronchoconstrictor fibres; rather is it put forward as an alternative mechanism by which bronchospasm may develop. Since the classical researches of Longet [1842], MacGillavry [1877], Einthoven [1892], Brodie and Dixon [1903], and Dixon and Ranson [1912] demonstrating that bronchoconstriction may be produced by electrical direct or reflex stimulation of the vagus, speculations as to the cause of asthmatic attacks have largely centred around vagal mechanisms rendered unduly sensitive by a hormonal imbalance, a neuropathic or allergic diathesis, and other factors which are too numerous to mention here. Clinical observations, however, and the failure of atropine to alleviate many cases of bronchospasm, do not always support the interpretation that the parasympathetic nervous system is primarily involved in asthmatic attacks of nervous origin. In this connexion one point of importance may be interposed. The failure of atropine to alleviate bronchospasm in certain cases may not be unequivocal evidence that activity of the cholinergic bronchoconstrictor fibres is not responsible for the bronchospasm. It may be that insensitivity of the bronchial muscles to atropine is inherent in an asthma diathesis. Now, we have clearly demonstrated in a large number of experiments that single injections of adrenaline cause bronchodilatation in freshly perfused lungs, but bronchoconstriction after two hours of perfusion. Further, the early addition of adrenaline to the perfusate, either in the form of repeated injections or as an infusion, prevents the onset or diminishes the magnitude of adrenaline bronchoconstriction. To these experiments we give the tentative interpretation that lack of adrenaline in the blood is in part responsible for the bronchoconstriction which occurs as the result of single injections of adrenaline. We suggest that this phenomenon may have its counterpart in the human body in that adrenaline suddenly released by exercise or by an emotional stimulus—either from the suprarenal glands or at the termination of adrenergic fibres supplying the bronchial muscle—will cause bronchoconstriction in subjects suffering from hypoadrenalinæmia due to adrenal gland hyposecretion or to an abnormally high rate of destruction of adrenaline in the blood.

Appendix.

Method of Graphic Representation used in Figures.

In our series of experiments the changes in tidal air occurring over a perfusion period of several hours have been of two kinds: the first an immediate change due to the injection of a drug, and the second a gradual change due to a variety of factors, such as the "goodness" of the preparation, the steady infusion of drugs, the nature and dosage of single injections of drugs, as well as the interval of time between single injections. The necessity for some method to enable a rapid comparison to be made of the effects on the tidal air of a drug injected several times during a prolonged perfusion, with a changing background, became evident at the outset of the investigation. The necessity became more urgent when it was desired to compare the results obtained from an increasing number of experiments. After trying several methods of plotting the tidal air changes, the graphic representation described below was devised in conjunction with Dr. A. Brown and found to be the most suitable.



Graphic representation of the tidal air responses to adrenaline, 2·0 μg. at arrow 3, 10·0 μg at 4, and to acetylcholine, 2·0 μg at arrow 6 and 20·0 μg. at 7. See text.

The method is best described by illustrating the graphic representation of the tidal air responses to the injection of two drugs. In the figure, A represents a portion of the tidal air tracing on the kymograph paper, in which 2·0 μg. of adrenaline or of acetylcholine are ineffective, but 10·0 μg. of adrenaline and 20·0 μg. of

although weakening our thesis, may prove helpful in planning future investigations. Further, it is not to be supposed that an asthmatic attack follows an emotional stimulus in every individual suffering from hypoadrenalinæmia. The unknown diathesis factor must presumably be present and, on our hypothesis, so render the bronchial muscle abnormally responsive to a sudden rise in adrenaline concentration in the blood. This condition is simulated in the perfused lung.

Summary.

1. A method is described for the separate blood perfusion of each *lung under negative pressure ventilation,* whereby *one lung can be used as a control, the other as a test-object.*

2. In isolated lungs perfused with defibrinated or heparinised blood, the bronchial response to injections of adrenaline (2–10 μg.) repeated at ten-minute intervals over a period of three hours varies according to the duration of perfusion. The first injection in freshly perfused lungs causes bronchodilatation. Injections during the succeeding 40–90 minutes produce a progressively weaker bronchodilatation, or are without effect. Subsequent to this period adrenaline injections cause a moderate bronchoconstriction.

3. The bronchoconstrictor response fails to appear if during the earlier period of perfusion adrenaline is steadily infused into the circulation in quantities greater than 5 μg./min. or if too frequent and large single doses of adrenaline are given.

4. In lungs perfused for two hours without adrenaline being added to the blood, the first injection of adrenaline thereafter generally causes a powerful bronchoconstriction. The adrenaline bronchoconstrictor response appears in *nicotinised* and *atropinised* lung preparations, but is suppressed or reversed by ergotoxine.

5. Once the adrenaline bronchoconstrictor response has appeared, its subsequent appearance to single doses of adrenaline depends upon the dose administered and the previous "adrenaline history" of the preparation.

6. In some perfused preparations the pulmonary arterial pressure rise to adrenaline is markedly increased after 120 minutes of perfusion as compared with the effect of adrenaline on the freshly perfused preparation.

7. No evidence has been obtained that the addition of posterior pituitary extracts affects the occurrence of adrenaline bronchoconstriction.

8. The possible significance of the results enumerated above is discussed in relation to potential mechanisms governing the onset and alleviation of certain types of asthmatic attacks.

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acetylcholine produce bronchodilatation and bronchoconstriction respectively. If these T.A. changes were merely plotted as percentile changes of the initial tidal air value the graph shown at B would be obtained. We found that such a graph depicting some 20 injections of drugs, some of which produced only weak immediate tidal air changes, did not enable, at a glance, a differentiation to be made between these immediate effects and the gradual changes in tidal air due to other causes.

The description of the new method by which the graphic representation C is derived from the tidal air changes shown at A will be more easily understood if at first no attention is paid to the lines joining the various points. At arrows 1 and 2 in A the initial value of the T.A. remains unchanged and is taken as 100 p.c.: the corresponding points on graph C are surrounded by a circle to indicate that they do not represent T.A. values immediately either before an injection or after the maximum response of the bronchi. They will be referred to as interval points. At arrow 3, 2·0 μg. of adrenaline caused no change in the T.A. and therefore the corresponding point on graph C *falls on the 100 p.c. line. At arrow 4, 10·0 μg. of adrenaline increased* the T.A. to 200 p.c. of its initial value. A point is placed on the 100 p.c. line to correspond with the T.A. value immediately before injection, and a second point on the 200 p.c. line, *on the same ordinate*, to indicate the maximum response. Thus the vertical distance between the two points on the injection ordinate is proportional to the degree of T.A. response. At arrow 6, 2·0 μg. of acetylcholine was ineffective, and therefore the corresponding point on graph C again lies on the 200 p.c. line. At 7, 20·0 μg. of acetylcholine reduced the T.A. to 150 p.c. of its initial value, and therefore two points are placed on the injection time ordinate, one at the 200 p.c. level and the other at the 150 p.c. level. Finally, an interval point is represented at 9.

The next step involves joining up both points which lie on the same ordinate to the adjacent points, which may be either an interval point or one denoting an effective or an ineffective injection. When this has been done a series of areas are obtained on the graph: those areas resulting from bronchodilatation are left unshaded, those resulting from bronchoconstriction are filled in black. This procedure has the effect of putting forward the response by the distance between two points of measurement, but this discrepancy is of little account provided it is realised that the injection ordinate illustrates the nature and magnitude of the response.

When a complex T.A. response occurs, such as bronchodilatation succeeded by bronchoconstriction, the two parts of the response are treated separately. If the maxima of the double response were both placed on the injection ordinate there would be no indication of the nature of the response. To overcome this difficulty the second maximum is not plotted on the injection ordinate, but is plotted a little later in time, so that, when the various points are joined up, two areas indicate the T.A. change.

The P.A.p. kymograph record is graphed by a similar method.

CLEARANCE AND CUMULATION OF ADRENALINE. By M. Y. ANSARI. From the Department of Pharmacology, University of Edinburgh.

(*Received for publication 30th September 1941.*)

INTRODUCTION.

ADRENALINE produces a transient action and it is generally assumed that the drug is inactivated in the body by some enzyme. Adrenaline *in vitro* is oxidised by various inorganic catalysts and by a variety of oxidases; in particular by catechol oxidase, by the cytochrome-cytochrome-oxidase system, and by the amine oxidase. Richter [1940] concluded that the chief method of inactivation in the human body was by conjugation with sulphates.

The amine oxidase shows certain suggestive peculiarities as regards its susceptibility to enzyme poisons. It is cyanide resistant, is inhibited easily by narcotics, is not inhibited by anti-oxidising agents such as ascorbic acid but is inhibited by ephedrine and cocaine.

The phenol oxidase is inhibited by cyanide and ascorbic acid, but is not inhibited by ephedrine and cocaine. The majority of these properties agree with the hypothesis that adrenaline is inactivated in the body by the amine oxidase.

Against this hypothesis there are two major objections: firstly, that the rate of action of amine oxidase on adrenaline *in vitro* is far slower than the rate of inactivation of adrenaline in the body, and secondly, that no amine oxidase can be demonstrated in certain tissues, such as the rabbit's ear, which are thought to inactivate adrenaline [Gaddum and Kwiatkowski, 1938, and Richter, 1940].

The liver is the chief tissue which contains considerable quantities of amine oxidase and hepatectomy does not alter the rate of inactivation of adrenaline in dogs [Markowitz and Mann, 1929].

Similar objections can be raised against the hypothesis that catechol oxidase inactivates adrenaline. Some plants contain large amounts of this enzyme, but the activity of mammalian tissues in this respect is very much less than plant tissues [Duchateau-Bosson and Florkin, 1939]. Recent accounts of this controversial subject are given by Philpot [1940] and Blaschko and Schlossmann [1940]. Clark and Raventos [1939 *b*] showed that the isolated auricle of the frog inactivated adrenaline and that this effect was inhibited by ascorbic acid. This would agree with the hypothesis that the inactivation was due to

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cytochrome-oxidase which is known to be present in all tissues. De Meio and Luduena [1940] found, on the other hand, that the dog's retractor penis inactivated adrenaline slowly (10–50 per cent. in 2–5 hours) and that this action was not inhibited by cyanide (1–2 times 10^{-3} molar), a concentration which would certainly inhibit cytochrome-oxidase.

Clark and Raventos [1939 *b*] estimated the rate of inactivation of adrenaline in the cat by measurements of the dosage-duration curve. They found that half-clearance occurred in about 20 sec. when the dosage was below 3 μg./kg., but that with larger doses the rate of clearance was much slower; the results suggested enzyme saturation with a constant removal of 0·1 to 0·2 μg./sec.

The experiments described in this paper were undertaken in order to determine by means of continuous intravenous infusion the nature of the mode of clearance of large doses of adrenaline.

The general principles of the tests made are as follows: When an enzyme acts on a substrate that is not present in large excess the destruction usually follows a unimolecular course and a constant fraction of the amount present is broken down per minute ($C_t = C_0 e^{-at}$. C_t = concentration at time t, C_0 = original concentration, a = clearance constant). In such a case continuous infusion of a drug will result in a rise in the concentration of the drug present until the fraction broken down per minute equals the amount introduced per minute, and after this equilibrium occurs. The course of cumulation follows the formula

$$C_t = \frac{b}{a}(1 - e^{-at}),$$

where b = rate of introduction. When t is large e^{-at} becomes very small and the formula becomes

$$C_t = \frac{b}{a}.$$

If, for example, $a = 1/20$ per min., then equilibrium will occur when the amount cumulated equals 20 times the amount introduced per minute.

If a clearance process such as has been described occurs, then a series of different rates of introduction will result in plateaus of cumulation at a series of different levels.

If, on the other hand, a constant quantity is removed per minute, then a plateau will only be attained when the amount introduced per minute equals the amount cleared, and any increase in the rate of introduction will result in steady cumulation of effect.

Methods.

The experiments were performed on cats either with the brain and spinal cord destroyed or narcotised with chloralose and ether. The

left nictitating membrane was used in all experiments. The vagi were cut, the left superior cervical ganglion removed, and the suprarenal glands were either removed or ligated.

Dosage-Duration Relations.

In the case of a drug cleared by enzyme action the clearance should follow the formula $C_t = C_0 e^{-at}$. The durations of action with different

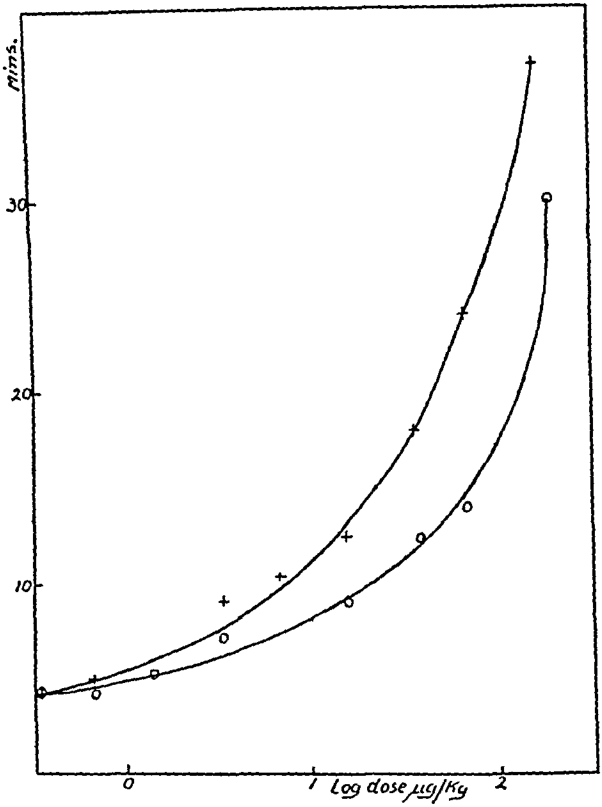

FIG. 1.—Dosage-duration relation of responses of cat to adrenaline injections (averages from 20 experiments). Abscissa: log. dose µg./kg. Ordinate: duration of response in minutes. Circles: blood-pressure; crosses: nictitating membrane.

doses may be assumed to measure the time until the concentration falls to a certain threshold value, hence $C_0 . e^{-at} =$ constant. This implies that t varies as log. dose.

Clark and Raventos [1939 *b*] found this to be true with small doses (less than 3 µg./kg.) of adrenaline, but not to hold with large doses.

Our results show the same relation (fig. 1). When the dose rises above 10 µg./kg. the durations are much longer than are to be expected from the formula given. The durations measured were two or three

times as long as those measured by Clark and Raventos, but the general shape of the curves is the same in the two sets of experiments. The times of half-action for various doses can be measured from the tangents of the curves in fig. 1. According to the formula given above the factor a equals 0·7/time half-action.

Cumulation with Continuous Infusion.

Many authors have shown that infusion of adrenaline at a constant rate will maintain a response at a constant plateau for long periods [Trendelenburg, 1924; Dragstedt *et el.*, 1928; Prohaska *et el.*, 1937].

In order to estimate the cumulation of adrenaline produced when a plateau was attained by continuous infusion, the cats were standardised by a series of quick injections and the continuous injections were interspersed.

The nictitating membrane was more satisfactory than the blood-pressure for this type of experiment, because the former maintained a steady plateau with continuous infusion, whereas the latter usually attained a maximum and then declined.

The sensitivity of the cats varied over a fivefold range. Fig. 2 shows collected results obtained on the nictitating membrane with 4 sensitive and 2 insensitive cats.

The dosage response curves follow the rectangular hyperbola found by many authors [Bacq, 1935; Rosenblueth, 1932; Clark and Raventos, 1939 *b*]. The curves are drawn to the formula that expresses a reversible unimolecular reaction

$$x \,.\, 1/k = \frac{y}{150 - y},$$

where 150 equals the maximum action. The values of k are 10 and 31 μg./kg.

In all cases the continuous injection resulted in a plateau being attained. The graph shows that all responses to continuous infusion were below the maximum response, hence the plateau was not due to the tissue having contracted to the maximum extent. This point was confirmed in the case of high doses by giving a quick injection of adrenaline in addition to the continuous flow, and this caused an extra contraction in all cases.

The fact that a plateau response was attained with large doses (10 μg./kg./min.) indicates that the rate of clearance was increasing with the amount present as the result of these rates of injection. Hence the conclusion of Clark and Raventos [1939 *b*] that enzyme saturation occurs with doses over 3 μg./kg. is probably incorrect.

It seems reasonable to assume that the occurrence of a plateau with continuous infusion indicates a concentration of adrenaline equal to

that which produces the same effect when given as a quick injection. If this assumption be allowed then the rate of destruction can be calculated, provided there is no large difference in distribution in the two cases.

The plasma volume of a cat is between 5 and 10 per cent. of the body weight, and the extracellular fluids are about 25 per cent. body weight. A quick injection of adrenaline will reach the susceptible tissue before it has mixed with the whole of the blood. Therefore, if

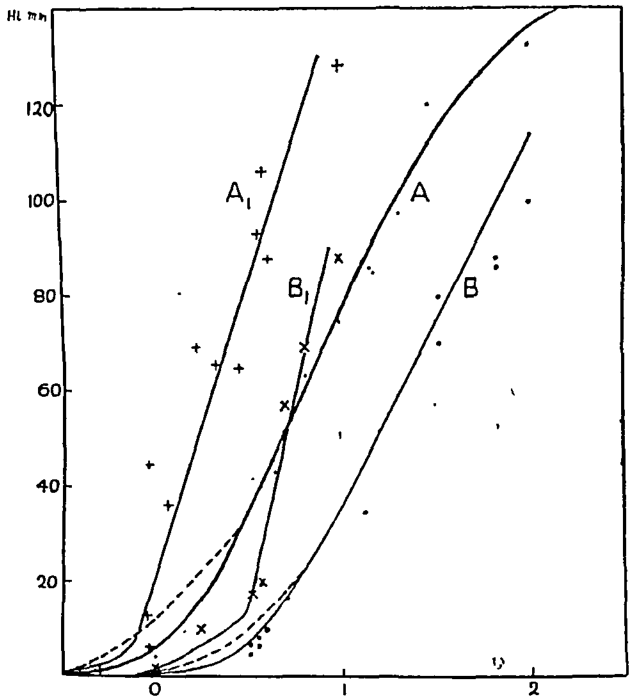

FIG. 2.—Effects produced by quick (A and B) and continuous (A_1 and B_1) doses of adrenaline. A and A_1, figures from 4 sensitive cats; B and B_1, figures from 2 insensitive cats. Abscissa: log. dose µg./kg. or µg./kg./min. Ordinate: height of response of nictitating membrane. The dotted line shows where the curve drawn to the formula $x.1/k = y/100 - y$ deviates from the observed values.

1 µg./kg. be given, the tissue is exposed for a few seconds to a concentration of about 20 µg./kg. Continuous infusion provides plenty of time for equilibrium to be attained with the tissue fluids, hence a dose of 1 µg./kg. spread over a minute is unlikely to produce a concentration of more than 4 µg./kg.

Experiments were made to determine the extent to which this source of error affected our results. A series of doses were given alternately as quickly as possible and spread over a minute. The responses of the blood-pressure were equal under the two conditions, whilst the responses of the nictitating membrane were about 25 per cent. less

with the slow injection. These results indicate that the ratio of equiactive doses with slow and quick injection is certainly less than 2 to 1 and may be equal to 1 : 1.

The interval between quick injection and maximum response with both N.M. and B.P. was 0·5 to 3 min., whilst the response to continuous infusion attained a plateau in about 5 min. Adrenaline therefore does not act with great rapidity, but quickly attains a plateau with continuous injection, hence differences of distribution with the two forms of injection are not important.

The blood-pressure responses were not as satisfactory for measurement as were those of the nictitating membrane, but in general showed the same relation between dosage and height of plateau response. The ratio quick dose (μg./kg.)/continuous dose (μg./kg./min.) was nearly unity with doses near the threshold and rose to about 5 with large doses.

Relation between Quick and Continuous Dosage.

Fig. 3 shows averages of results obtained in 16 experiments. The relation between the two forms of dosage is that continuous dose

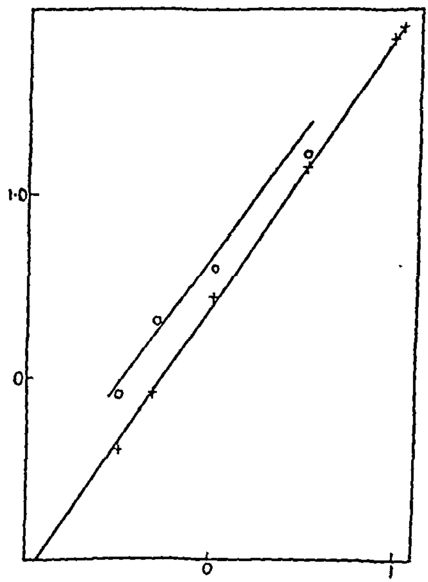

FIG. 3.—Relation between log. continuous dose μg./kg./min. (abscissa) and log. quick dose μg./kg. of adrenaline (ordinate), which produce equal effects. Average figures from 16 experiments. Crosses: nictitating membrane; circles: blood-pressure.

varies as (quick dose)$^{0 \cdot 66}$. The amount of drug introduced per minute when the plateau is attained equals the amount removed and the quick dose indicates the amount cumulated, hence the figure sho

the relation between the concentration of drug present (x) and the amount removed per minute (continuous dose). The relation found indicates, therefore, a clearance by a process, probably enzymatic, in which the amount of substrate changed per minute varies as (substrate conc.)$^{0\cdot66}$. The fraction of substrate present that is cleared per minute $(a)=\frac{b}{x}$, and the value of a can only be constant when b varies as x. In this case b varies as $(x)^{0\cdot66}$; hence

$$a=k\,.\,\frac{x^{0\cdot66}}{x}=k\,.\,\frac{1}{(x)}0\cdot33$$

or a is inversely proportional to $\sqrt[3]{x}$. Fig. 4 shows the relation between a and x calculated by two completely independent methods: (1) from

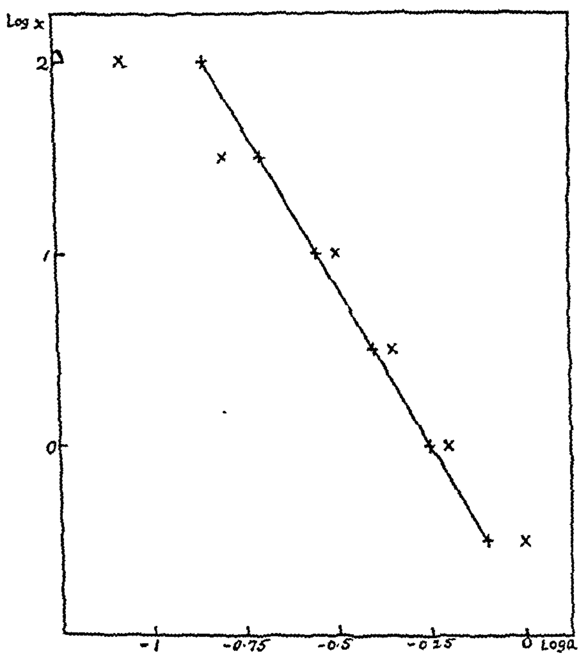

FIG. 4.—Relation between concentration of adrenaline (x) and fraction cleared per min. (a).
Abscissa: log. a; ordinate: log. x.

Log a calculated from × Dosage-duration relations.
" " " + Relation between rate of continuous infusion and the height of plateau response.

the dosage-duration relation with quick injections, and (2) from the relation between the rate of continuous infusion and the height of the plateau response. The two sets of calculations agree fairly well.

with the slow injection. These results indicate that the ratio of equiactive doses with slow and quick injection is certainly less than 2 to 1 and may be equal to 1 : 1.

The interval between quick injection and maximum response with both N.M. and B.P. was 0·5 to 3 min., whilst the response to continuous infusion attained a plateau in about 5 min. Adrenaline therefore does not act with great rapidity, but quickly attains a plateau with continuous injection, hence differences of distribution with the two forms of injection are not important.

The blood-pressure responses were not as satisfactory for measurement as were those of the nictitating membrane, but in general showed the same relation between dosage and height of plateau response. The ratio quick dose (μg./kg.)/continuous dose (μg./kg./min.) was nearly unity with doses near the threshold and rose to about 5 with large doses.

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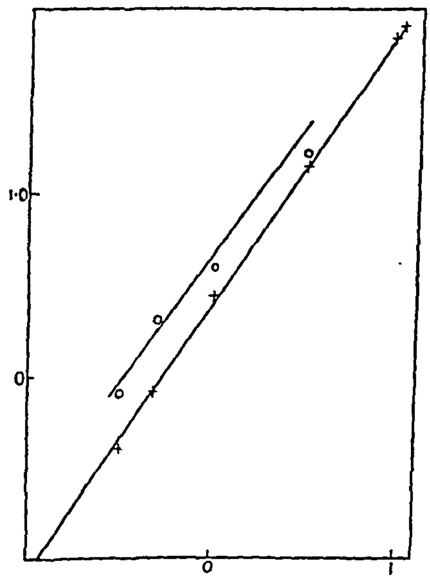

FIG. 3.—Relation between log. continuous dose μg./kg./min. (abscissa) and log. quick dose μg./kg. of adrenaline (ordinate), which produce equal effects. Average figures from 16 experiments. Crosses: nictitating membrane; circles: blood-pressure.

varies as (quick dose)$^{0·66}$. The amount of drug introduced per minute when the plateau is attained equals the amount removed and the quick dose indicates the amount cumulated, hence the figure shows

TABLE I.

Adrenaline dosage—							
(a) Continuous μg./kg./min.	0·34	0·34	1·02	3·4	5·0	6·8	10·2
(b) Additional quick injection μg./kg.	1·0	3·4	3·4	10·2	10·2	10·2	10·2
Response on n.m. after (a) .	0·5	0·5	2	17	57	69	88
(b) .	2	7·5	12	51	83	91	101
Adrenaline equivalents of (a)	0·5	0·5	1·2	3·8	19	28	60
(b)	1·2	2·2	3·0	14	50	70	110
Difference	0·7	1·7	1·8	10·2	31	42	50

adrenaline that was given, but with larger doses the calculated dose was 3 to 5 times as great as the dose given.

Table II. shows figures calculated in a similar manner from 5 experiments. With continuous doses less than 5 μg./kg./min., which

TABLE II.

Continuous dose, μg./kg./min.	Additional dose, μg./kg.	Value of additional dose, μg./kg. Calculated from response.
0·33	1·02	1·5, 1·0
	3·4	1·7
0·5	1·5	0·4, 2·5
	5·0	5·0
0·66	3·3	2·3
1·0	3·4	3·5, 3, 1·0
	5	7
1·5	15	18
1·66	6·6	8·7
2·66	10	16
3·4	10·2	10
4·0	10	15
5·0	10	29
	15	>100
6·8	10	45
10	10	40
	15	10
15	49	64

produce a response of one-quarter the maximum or less, the figures calculated from the response to the additional injection are approximately correct, but when larger doses were given with continuous injection the added response was much greater than was to be expected.

Blood-pressure responses also were measured and these gave similar results with low rates of continuous infusion, but with higher rates the plateau was not maintained at a constant level, and hence quantitative estimates of the additional response could not be made.

The most probable reason for the excessive responses produced by

Additive Effects.

The validity of the method used depends on the assumption that the production of a plateau response of a certain height indicates that a certain concentration of adrenaline is present in the blood stream.

This assumption was tested by giving continuous infusions until plateaus were attained and then adding a quick injection of adrenaline. Fig. 5 shows the results obtained in one experiment. The continuous line and dots show the relation between dosage and the response of the nictitating membrane to quick injections of adrenaline. The crosses

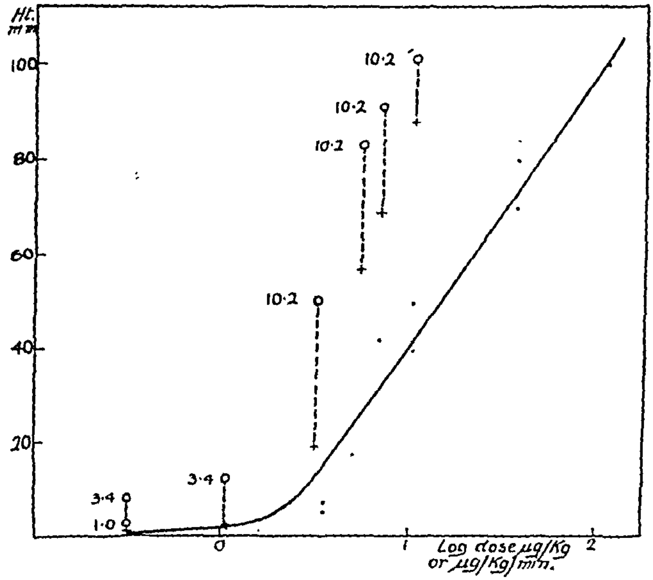

FIG. 5.—Effects produced by quick injections of adrenaline superimposed on continuous infusion. Abscissa: log. dose µg./kg. or µg./kg./min. Ordinate: height of response of nictitating membrane. Continuous line and dots: dosage-response curve to quick injections. Crosses: response to continuous injection; figures and circles: amount of superimposed quick injection and height of response.

show the heights of plateau produced by continuous infusions at the rates indicated by the abscissæ.

The dotted lines, circles and numbers indicate the extra response produced by quick injections of adrenaline given during the continuous administration. The adrenaline equivalents (µg./kg.) to the crosses and circles can be read off from the dosage/response curve. Table I. shows how the effect of the additional injection was calculated from the results given in fig. 5.

With low doses of adrenaline the difference between the adrenaline equivalents of the responses is similar to the additional quick dose of

TABLE I.

Adrenaline dosage—							
(*a*) Continuous μg./kg./min.	0·34	0·34	1·02	3·4	5·0	6·8	10·2
(*b*) Additional quick injection μg./kg.	1·0	3·4	3·4	10·2	10·2	10·2	10·2
Response on n.m. after (*a*)	0·5	0·5	2	17	57	69	88
(*b*)	2	7·5	12	51	83	91	101
Adrenaline equivalents of (*a*)	0·5	0·5	1·2	3·8	19	28	60
(*b*)	1·2	2·2	3·0	14	50	70	110
Difference	0·7	1·7	1·8	10·2	31	42	50

adrenaline that was given, but with larger doses the calculated dose was 3 to 5 times as great as the dose given.

Table II. shows figures calculated in a similar manner from 5 experiments. With continuous doses less than 5 μg./kg./min., which

TABLE II.

Continuous dose, μg./kg./min.	Additional dose, μg./kg.	Value of additional dose, μg./kg. Calculated from response.
0·33	1·02	1·5, 1·0
	3·4	1·7
0·5	1·5	0·4, 2·5
	5·0	5·0
0·66	3·3	2·3
1·0	3·4	3·5, 3, 1·0
	5	7
1·5	15	18
1·66	6·6	8·7
2·66	10	16
3·4	10·2	10
4·0	10	15
5·0	10	29
	15	>100
6·8	10	45
10	10	40
	15	10
15	49	64

produce a response of one-quarter the maximum or less, the figures calculated from the response to the additional injection are approximately correct, but when larger doses were given with continuous injection the added response was much greater than was to be expected.

Blood-pressure responses also were measured and these gave similar results with low rates of continuous infusion, but with higher rates the plateau was not maintained at a constant level, and hence quantitative estimates of the additional response could not be made.

The most probable reason for the excessive responses produced by

quick doses after large continuous doses is that the latter saturate the tissue fluids and cells with adrenaline and hence check the diffusion out of the plasma of the quick doses.

In calculations of this kind the fact that the dosage-response relation follows a hyperbola can produce very misleading results unless caution is exercised. For example, fig. 5 shows the following relation between rate of continuous injection and the height of response to a constant quick injection of adrenaline.

Continuous injection μg./kg./min. .	0	3·4	5·0	6·8	10
Height of response (mm.) to 10 μg./kg.	42	34	26	22	13
Adrenaline equivalent of rise calculated from dosage-response curve.	10	10	31	42	50

The larger the continuous injection the smaller is the amplitude of the response to the quick dose, but if the adrenaline equivalents of these responses be calculated from the dosage-response curve it is found that this figure rises as the amount of continuous injection is increased.

Measurement of the amplitude of response without considering the dosage-response relation would lead to the conclusion that continuous adrenaline inhibited the action of quick injections of the drug and this might be produced as a proof of the "potential action" of adrenaline. In reality, the reverse is the truth and the continuous administration increases the effect produced by the quick injection. This provides a simple example of the errors that can occur in calculating synergisms or antagonisms of drug action.

Form of Response to Cumulative Dosage.

Fig. 6 shows the response of the n.m. to continuous infusion of 3·4 μg./kg./min. for 10 min. The right ordinate shows the adrenaline equivalents (μg./kg.) calculated from the dosage-response curves.

The plateau height indicates half destruction in 2·9 min. The cumulation and disappearance calculated from this figure are shown by curves B and C. Curve A shows the response which should occur if there were no clearance.

The curves show clearly that the plateau effect is not due to a maximum response having been produced. The recovery after cessation of dosage agrees well with the calculation of the dose cumulated. The rate of rise of the response is more rapid than that calculated, and this error would be increased if allowance were made for the fact that the rate of clearance is not constant but decreases as the adrenaline accumulates. There are so many variables that affect the rate of action of drugs that it is not profitable to try to explain this discrepancy.

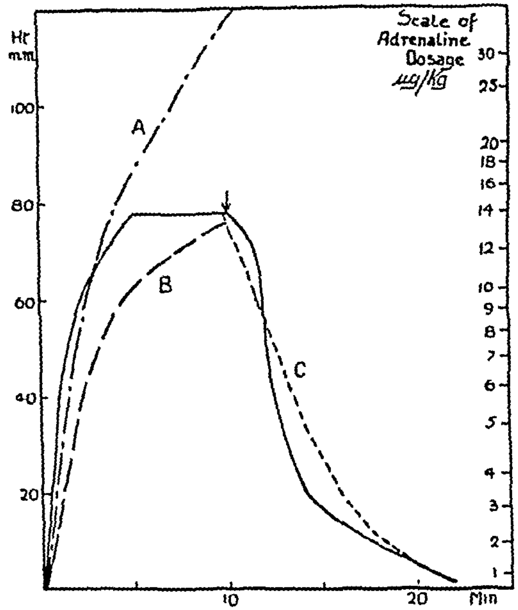

FIG. 6.—Observed and calculated course of response to continuous injection of adrenaline. Abscissa: time in minutes. Ordinates: left, height response n.m.; *right, adrenaline dosage producing corresponding height of response.* Continuous line: response produced by 3·4 μg./kg./min. adrenaline for 10 min. Dotted lines: (A) calculated response if no clearance; (B) calculated response if half-clearance in 2·9 min. ($a = 0{\cdot}24$); (C) calculated fall at this rate of clearance.

DISCUSSION.

The most important fact shown by the results is that the response to continuous adrenaline administration attains an equilibrium lower than the maximum responses, even when the adrenaline dosage is large.

The results also show that the adrenaline clearance does not follow the simplest or unimolecular type of enzymatic clearance, in which the fraction of drug present that is cleared per minute is constant. In the case of adrenaline this fraction decreases as the concentration rises. The relation found is that the amount of adrenaline cleared per minute varies as $(\text{conc.})^{0{\cdot}66}$, and the fraction cleared per minute varies as $1/(\text{conc.})^{0{\cdot}33}$. The fractions cleared per minute (a) varied from 0·83 with 0·3 μg./kg. to 0·13 with 100 μg./kg. These values correspond to times of half-clearance of 0·85 min. and 5·4 min.

These times of half-clearance are somewhat longer than those calculated by Clark and Raventos, which ranged from 0·3 to 3 min. These times are, however, much shorter than the rates of destruction found with the amine oxidase [Richter and Tingey, 1939]. The rates of destruction calculated from the plateaus produced by continuous infusion agree with the rates calculated from the dosage-duration relations.

These results can be explained most easily on the assumption that the adrenaline is removed by some form of enzymatic action. The rates of removal even with large doses are, however, much more rapid than the rates of removal found with the amine oxidase. If the removal is due to this enzyme then its action *in vivo* must be much more rapid than its action *in vitro*.

The rates of removal calculated in the present paper are somewhat slower than those calculated by Clark and Raventos [1939 *b*]. This increases a difficulty encountered by these authors [1939 *a*], who found that the adrenaline liberated by sympathetic stimulation in the cat was reduced to half-concentration in 5 to 20 seconds, which was a shorter time than that expected from their calculations of the rate of adrenaline clearance.

Summary.

1. Continuous infusion of adrenaline in the cat produces over a wide range of dosage a plateau response in both the nictitating membrane and blood-pressure.

2. This indicates that enzyme saturation does not occur even with large doses [correction of conclusion of Clark and Raventos, 1939 *b*].

3. The rate of clearance of adrenaline decreases as the concentration is increased. The relation found is that the fraction cleared per minute varies inversely as $\sqrt[3]{\text{(conc.)}}$.

4. The relation between dosage and response and the relation between rate of continuous infusion and height of plateau response indicate similar rates of clearance.

5. The times of half-clearance found varied from 0·8 to 5 minutes.

I gratefully acknowledge my indebtedness to the late Professor A. J. Clark for his help and valuable suggestions throughout the course of this work. I also wish to thank Dr. J. M. Robson for going through the written matter, and Mr. N. E. Condon for the technical help.

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SOME EFFECTS OF THYROID AND GONADOTROPHIC PREPARATIONS IN THE FOWL. By ALAN W. GREENWOOD and J. S. S. BLYTH.

From the Institute of Animal Genetics, University of Edinburgh.

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WITSCHI [1935] has shown that certain pituitary and allied preparations can induce maturation and even full activity in the resting gonads of sparrows and finches. But while hormonal control appears to be involved in the non-breeding season, as it is in the œstrous cycle of mammals and the brooding cycle of birds, there is general agreement that the initiation and termination of the quiescent period are also related to certain external stimuli such as light variations. The researches of Bissonnette and Benoit indicate that these external agents require the intermediation of the anterior pituitary in order to exert their effect [reviewed by Marshall, 1936; Van Dyke, 1939, p. 58].

As a rule, the annual autumnal cessation of egg production in the fowl coincides with the onset of the moulting period, and it might be assumed that the two processes are mutually incompatible, or too expensive physiologically to be carried on together. These views are supported by evidence from an inbred line, maintained at this Institute, in which a number of females have occurred which laid for two consecutive years without a break; such birds either did not moult, or replaced only a small percentage of their feathers while laying at a reduced rate.

Nevertheless, the length of the infecund period varies greatly in individuals, and often far exceeds the time required for the completion of feather replacement. In a group of 58 hens, drawn from hatches in three successive years, the length of the gap at the end of the first laying year was 84·8 days, with a standard deviation of 34·56 days. The gap also lengthens with age: for the same 58 birds the mean difference between the dates of renewal of production in the second and third years is 20·29 days (S.E., 3·89), and between the second and fourth years 29·74 days (S.E., 4·03). Greenwood *et al.* [1940] showed that although individual birds appeared to have a genetically determined date on which production ended, second-year birds tended to cease laying slightly earlier than pullets. In the same communication it was also pointed out that the range of dates on which birds cease to

lay is very wide: it varied from July to December in the stock examined. If, therefore, external agents are basically responsible for the change-over from production to moulting, it is obvious that they do not act alone, but are dependent on the presence of certain suitable conditions within the organism itself.

The present report deals with the results of some preliminary investigations of the effect of anterior pituitary extracts and allied preparations, and of thyroid, administered to hens in and about the moulting period.

Material.

The gonadotrophic preparations used were provided through the kindness of the Medical Research Council. They were Pregnant Mare's Serum Q2, 30 I.U. per mg. (P.M.S.); Chorionic Gonadotrophin U.P. 31, 100 I.U. per mg. (U.P.), and three extracts of anterior pituitary, viz. Sheep A.P. 117B, Horse A.P. 118B, and Pig A.P. 74D; the biological activity of the pituitary fractions is discussed by Chance *et al.* [1939]: "B" signifies the alkaline soluble portion of acetone desiccated tissue, and "D" the fraction of "B" soluble in aqueous solution between pH 3 and pH 6. The thyroid used was the B.D.H. commercial product "Thyroideum."

The hens were part of the normal breeding stock of Brown Leghorns maintained at the Institute, and were aged from 2 to 4 years.

The gonadotrophic preparations were introduced in three ways: (1) injections of a 10 mg. per c.c. aqueous solution, (2) subcutaneous implants of pellets in which the excipient employed was glucose, and (3) pressure tablets. The thyroid was administered in gelatin capsules until they were no longer available, when it was fed as pills made up with a little wet mash.

Experimental.

Gonadotrophic Preparations.—For 15 days from 3rd September 1940 the five preparations were administered to a like number of pens of hens at the rate of 1 mg. per bird per day; each pen contained two age groups. The majority of females in this stock begin to moult in September and October, but, as mentioned in the introduction, the time at which individuals commence varies widely, and to control the tests by comparison with untreated animals would not be satisfactory. The best estimate of the date would be the average for previous moults, but as the number of these varies from one to three, according to age, the data have been taken from the last year only: averaged figures show that the group receiving P.M.S. ceased production $33{\cdot}78 \pm$ S.D. $16{\cdot}87$ days after 1st September 1939; those receiving U.P., $43{\cdot}20 \pm 34{\cdot}62$; Pig Pituitary, $31{\cdot}25 \pm 19{\cdot}18$; Sheep Pituitary, $33{\cdot}37 \pm 20{\cdot}02$, and Horse Pituitary, $24{\cdot}14 \pm 8{\cdot}47$ days.

Apart from the group getting Horse Pituitary, the moulting dates in each pen are widely scattered, and some would have been expected normally to fall within the period of the test. Actually 7 birds, distributed over 3 groups, had already stopped production, though 4 of them laid within a week of the beginning of the experiment (Table I.). Since the comb regresses during ovarian inactivity, comb size (length + height) was used to indicate the course of changes in this organ. The results are summarized in Table I. No reaction was detectable in the groups receiving U.P., Sheep and Horse pituitary extracts. The mean

TABLE I.—GONADOTROPHIC EXTRACTS TO LAYING HENS.

1 mg. daily for 15 days.

	No. of birds.	Difference from original comb size at								No. of new birds moulting at		
		7 Days.		14 Days.		21 Days.		30 Days.				
		Mean mm.	S.E.	Mean mm.	S.E.	Mean mm.	S.E.	Mean mm.	S.E.	0 Days.	14 Days.	21 Days.
P.M.S.	8	4·50	0·87	5·50	0·87	− 9·00	2·01					
	7 *	3·29	0·68	1·71	0·84	−13·76	0·12					
	15 †	3·93	0·56	..	..	−11·43	1·32	−23·21	1·47	0	15	0
Pig Pituitary	16	3·06	0·42	2·00	0·81	− 3·31	2·06	− 7·81	2·43	0	2	7
U.P.	15	−4·73	2·01	−5·87	3·16	− 8·93	3·73	−13·07	4·08	4	4	0
Sheep Pituitary	17	−0·47	1·32	0·53	1·34	− 0·53	1·45	− 4·59	2·19	1	0	1
Horse Pituitary	8	0·12	1·41	−0·25	2·22	− 1·00	2·13	− 4·37	2·88	2	1	0

* 7 days' treatment only. † Total.

comb size of birds injected with Pig Pituitary rose slightly, then fell away normally after treatment ended, but neither egg production nor the number of birds entering the moult showed any abnormality. The only striking results came from the P.M.S. group: in it comb size increased noticeably during treatment; egg production ceased 1 to 5 days after injections began, except for one egg laid on the 15th day, and the hens passed immediately into a heavy moult. In half of this group the injections were not carried beyond the 7th day, and in these comb regression appears to have begun less abruptly. One bird was killed after 14 days' treatment and showed a normally active ovary with the three largest follicles degenerating. Although during treatment the hens continued to look as if in full lay, and possessed widely separated springy pelvic bones, none of them showed any inclination to nest after the 5th day.

The conclusion that P.M.S. was the only substance which definitely influenced the course of reproductive activity at this dosage-level was

further confirmed when all the moulting dates became available: the mean differences (with S.E.) between the dates in 1939 and 1940 in the various groups are as follows: P.M.S., $-27\cdot64 \pm 4\cdot64$ days; Pig Pituitary, $-3\cdot0 \pm 3\cdot52$; U.P., $1\cdot27 \pm 5\cdot18$; Sheep Pituitary, $10\cdot19 \pm 5\cdot19$; and Horse Pituitary, $2\cdot17 \pm 4\cdot09$ days.

Following the failure to maintain egg production beyond the normal moulting date, attention was turned to hens that had already ceased laying and the same substances administered, usually in heavier doses, in an attempt to bring them back to production. In an endeavour to obtain a prolonged subjection to the hormone, pellets made up with glucose, and compressed tablets of the pure substances, were implanted in the breast muscles in some cases.

As no really constructive indications had come out of the original experiments this set took the form of "feelers" carried out on single individuals or small numbers of birds. Periodic measurements made it possible to graph the course of each bird's comb regression and regeneration, and breaks in the smoothness of the curve indicated the effectiveness of the preparations tested. The details are summarized in Table II., and where fluctuations in comb size were small, and might be due to errors in measurement, the results have been tabulated as "no definite effect."

Here, as in the first tests, Horse and Sheep pituitary, and U.P. are without effect. P.M.S., even in higher doses, has less influence on comb behaviour than before; it may be capable of checking comb regression temporarily, but given alone is ineffective on fully regressed combs. When administered with thyroid there were indications of a renewal of comb growth. On the other hand, the indefinite action of Pig Pituitary in the earlier experiments is replaced by a marked positive effect when higher doses are used; irrespective of the state of the comb at the time of testing it usually responded to this extract by increasing in size. The increase, however, was always temporary, even under prolonged treatment, and though some of the birds appeared to return to a laying condition, with an enlarged comb and wide springy pelvic bones, they did not lay. The case of the bird autopsied suggests that maturation of the follicles, but no ovulation, was taking place.

Thyroid Feeding.—The fact that the comb showed growth when thyroid feeding was combined with the P.M.S. injections opened the question of whether the former would be effective alone, and 5 birds were fed 0·2 g. daily for 4 days; the combs began to increase sharply during treatment; when treatment ceased the increase continued in 3 at a slower rate, and in 2 the combs regressed again. After 10 days, medication with the same daily dosage of thyroid was recommenced in these and 3 other birds; the combs increased in size rapidly and by the 12th day all except one looked and handled as if in production. Hormone administration was stopped in 4 individuals at this point;

TABLE II.

	Given as	No. of birds.	Total per bird.	Duration treat-ment.	State of comb.*	Effect on comb.
			mg.	days.		
P.M.S.	Aqueous solution	1	5	1	I.	None.
	" "	1	3	1	R.	Regression inhibited 5
	" "	8	5	1	Min.	No definite effect.
	" "	3	14	8	Min.	None.
	" "	4	14 plus 0·8 g. thyroid in last 4 days	8	Min.	2 increased for 1 week, definite effect.
	Glucose pellet	2	20	..	R.	Regression inhibited 1
	Saline mush	2	10 plus 1 g. thyroid in 5 days	..	Min.	No definite effect.
	Pressure tablet	2	12	..	Min.	None; tablets slough in 9 days.
Pig Pituitary	Aqueous solution	1	91	19	R.	Increase 5 days, then r to rapid regression.
	" "	1	70	14	R.	6 days' increase, the gression. Killed 15th active ovary with sized degenerate yol
	" "	2	40	8	R.	6 days' increase, the gression.
	" "	4	20	5	Min.	Do.
	" "	3	70 plus 4 mg. œstrone in last 4 days	15	Min.	Do. (Œstrone ineffect
	Glucose pellet	2	20	..	R.	Regression inhibited 1
	Pressure tablet	1	12	..	Min.	Increased 1 week; regr
	" "	2	11	..	R. & Min.	None.
Sheep Pituitary	Aqueous solution	2	60	12	R.	No definite effect.
	Glucose pellet	1	20	..	Min.	None.
Horse Pituitary	Aqueous solution	1	25	5	R.	None.
	" "	2	40	7	Min.	No definite effect.
	" "	3	60	12	R.	None.
	Glucose pellet	2	20	..	R.	None.
	Pressure tablet	2	12	..	Min.	None; sloughed out days.
	" "	5	11–14·5	..	R.	No definite effect.
	" "	3	11–14·5	..	Min.	Do.
	" "	2	11–15·5	..	I.	Do.
U.P.	Aqueous solution	1	70	14	R.	None.

* State of comb at beginning of test.
I. = Increasing in size; R. = Regressing; Min. = Minimum, *i.e.* fully regressed comb.

1 laid the next day, and in the others the combs regressed. The 3 in which it was continued laid on the 16th, 19th, and 24th days, the first being one which had not had the previous 4 days' feeding; the combs levelled out a few days before they commenced to lay. Treatment was stopped immediately laying began, but as it seemed curious that

no deleterious effects resulted from its abrupt cessation 1 hen was given thyroid for a further 14 days; it also continued to lay normally.

Though none of these hens had shown any indications that comb growth was being renewed before the test began on 1st January, some had already passed the end of their previous moult gap, and there was just a chance that they might have come into production without thyroid medication. A second series of 7 were therefore selected, which had still some time to go before reaching their previous year's date for onset of laying. All the combs again responded immediately to a daily dose of 0·2 g., and 4 birds laid between the 18th and 31st day of treatment, dates which were from 13 to 36 days earlier than in the previous year. But, except in the case of the hen which laid on the 18th day, a peculiar retardation in the rate of comb growth began about the 11th day. The only obvious variation which could be related to this phenomenon was that the gelatin capsules in which the thyroid had been fed became unobtainable and from this time onwards the powder was fed as a pill made up with a little wet mash. In two individuals the comb became stationary and one began to regress despite continued feeding. Another pair which were showing practically no growth were given the addition of Pig Pituitary for 4 days in diminishing doses—5, 3, 2, and 1 mg. Though this caused renewed comb growth in both, and in one it continued until laying commenced at 26 days, regression supervened immediately in the other; similar diminishing doses of P.M.S. a week later produced the same results in this refractory individual. In the remaining two birds the check was not so pronounced and the comb continued its upward trend until they laid.

The impression gained from individual comb graphs was that growth in the thyroid-fed birds was accelerated beyond that in normals coming into production, and while the small numbers of the former, and the minor variations of treatment to which they were subjected, do not lend themselves to statistical examination, a rough comparison with untreated hens has been made. The steepness of the ascending comb curve varies from beginning to end, as in a normal growth curve, so that the larger the section considered the better, and though not all thyroid-fed birds were treated for quite this length of time, a three weeks' period has been chosen.

The rest of the hens in the original 5 pens were used as controls, and since the smallest increases resulting from thyroid feeding were 13 mm., untreated birds from all pens, making at least this amount of growth during the period of each test, were used for comparison (Table III.). In addition, the mean comb change for each of the original pens (excluding thyroid-fed birds) is given for the period of the first test. While the mean increase in thyroid-fed birds of both series is considerably higher than that in their respective

TABLE III.—AVERAGE COMB GROWTH FOR THREE WEEKS' PERIOD.

	No. of birds.	Increase mm. S.E.
Thyroid-fed birds, Series 1	8	27·87 ± 5·02
Untreated birds showing an increase of 13 mm. or more.	9	19·79 ± 2·04
Original group receiving P.M.S.	11	5·45 ± 1·44
,, ,, ,, Pig Pituitary	11	0·36 ± 2·04
,, ,, ,, U.P.	16	3·94 ± 2·82
,, ,, ,, Sheep Pituitary	17	1·88 ± 3·36
,, ,, ,, Horse Pituitary	6	0·50 ± 3·72
Thyroid-fed birds, Series 2	7	27·00 ± 3·31
Untreated birds showing an increase of 13 mm. or more.	16	23·31 ± 2·68

controls, the difference is not statistically significant in either case. The impression that they had a higher comb-growth rate is therefore not substantiated, though it seems possible that a positive difference might emerge with larger populations in the compared groups. It can be noted, however, that although all the hens in Series 1 and 2 show an increase of 13 mm. or more, only 9 out of 61 controls did so during the period of the first experiment and 16 during the second.

DISCUSSION.

In considering the negative nature of the results with some of the gonadotrophic preparations the question of the adequacy of the dosage arises. Certainly U.P. appears to have proved inactive in all previous tests on birds [Allen, 1939, p. 1027], but Witschi and Keck [1935] found Hill's extract of Horse Pituitary very effective in stimulating the quiescent ovary of the sparrow. Unfortunately, as these authors measure their doses in rat units, this does not allow of comparison with ours. In another communication, however, Witschi [1935] illustrated sparrow ovaries stimulated to complete development by daily doses of 2 RU of hypophyseal extract or 20 RU of Pregnant Mare's Serum. In the present experiments P.M.S. was active in the laying but not the non-laying hen, while similar quantities of Horse Pituitary extract were ineffective under both conditions. The report of Chance *et al.* [1939] indicates that Horse Pituitary is more active gonadotrophically (as tested in the immature rat) than either those from sheep or pig, yet a consistent positive effect was obtained with the last preparation in our fowls. Though further experimentation with different dosages is necessary to examine the adequacy of the amounts administered, it appears as if a species difference in relative reactivity to the various substances is present.

The failure of ovulation in stimulated ovaries and its actual inhibition in the hen by gonadotrophins have already been noted by Bates *et al.* [1935] and Walker [1925] respectively. On the other hand, Witschi [1935] obtained ovulation in both sparrows and finches by injections of P.M.S. Our results in laying hens with P.M.S. and non-laying birds with Pig Pituitary are in conformity with the former workers', and the alternatives arise that the opposing findings are due to species differences in reactivity, or that there is an optimum level of hormone capable of inducing ovulation, and lying within definite limits which were exceeded in all cases but Witschi's. Hartman's opinion [Allen, 1939, p. 630] that pre-ovulation cell differentiation is important for ovulation is in line with the latter suggestion. The further possibility of a qualitative difference in the substances used is beyond the scope of this paper.

Earlier researches on the effect of thyroid on ovarian activity are scanty and contradictory: Evans and Simpson [1930] and van Horn [1933] reported increased gonad-stimulating potency in the hypophysis of the hyperthyroid rat female, but Smelser [1934] found no increased potency in those of either the hypo- or hyperthyroid rat. From matings following a 3–5-day treatment of the female rat with thyroid substance, Kraatz [1939] obtained increased litter sizes in April, October, and December, but a deleterious effect supervened in hot weather. In fowls, Crew and Huxley [1923] were unable to demonstrate any effect of thyroid feeding on egg production, but Crew [1925] reported an increase in the production of senile hens under similar treatment.

Though ovulation was not obtained in all the thyroid-fed birds there is no doubt, from the behaviour of the comb, that this hormone had a definite stimulatory effect on the ovaries. The hens which failed to ovulate *under continued treatment* were all in the second series, where the change-over from administration in gelatin capsules to wet-mash pills was associated with a retardation in the rate of comb increase. While it has yet to be determined that this relation was not fortuitous, it may be that the gelatin possesses "augmentation" properties similar to those disclosed in other proteins, like casein and albumin, when added to gonadotrophins [Saunders and Cole, 1936]. A single bird, not belonging to the experimental series, proved refractory when fed thyroid in a wet-mash pill, but responded at once and laid in 10 days when the same daily dose was provided as a pill bound with a little jelly made from domestic gelatin.

The other point of note in this part of the experiment was that the abrupt cessation of feeding, either at the time of laying, or in one bird a fortnight after, was without effect on the continuance of ovarian function. Since administration of thyroid hormone is usually considered to depress the activity of the animal's own thyroid gland, this suggests that it is acting here not so much as a direct stimulatory agent

but rather as a releasing mechanism which allows the chain of events leading to ovulation to come into play. Nevertheless it has to be remembered that the initial stimulation was not enough, and that in birds which were not quite close to the point of production when treatment ceased the ovary returned to an inactive state. Thus it would have to be assumed that the hormone fed "carried" the factors producing ovarian activity until they were fully developed and became self-supporting. In the case of the only gonadotrophin which evoked a comparable effect—Pig Pituitary extract—stimulation of follicular development occurred but stimulation to ovulation was absent. Presumably the action was a direct one on the ovary, and any effect on the bird's own pituitary would be of a depressant nature, so that even had complete ovarian function ensued it would have been sustained at best only for so long as treatment lasted. Birds brought into lay by thyroid feeding, however, continued to do so, from which it may be inferred that this treatment instituted a regeneration of the gonad-stimulating hypophyseal activities which normally take place in the course of renewal of production. Benoit and Aron [1934], who found that thyroid stimulated the testes of young cocks and drakes, suggest that the different actions observed with various gonadotrophins may be due to differences in their thyreotrophic activity. In this connection it may be noted that Chance *et al.* [1939] found the Pig Pituitary the most active species thyreotrophically, which puts the assumption of a direct effect with this substance in question. With thyroid feeding there were no instances of birds remaining in an apparently laying condition without ovulation, but this does not entirely rule out the possibility that the thyroid gland was involved in the reaction to Pig Pituitary extract.

As a basis for further investigation along these lines our conception of the general endocrine situation in regard to the non-breeding season in the fowl may now be briefly discussed. Though the present results point to an insufficiency of thyroid activity, at least in the later part of the moult gap, Giacomini [1924] and Zavadowsky [1925] have shown that a single large dose of thyroid substance will precipitate a moult in the fowl. Hill *et al.* [1934] found that moulting followed hypophysectomy, but that the colour and structure of the new feathers were indicative of a marked hypothyroid condition. Greenwood [1936] concluded that moult is an inherent characteristic of all fowls irrespective of the presence or absence of sex glands, but that a certain degree of sexual activity is instrumental in inhibiting the moult. Seasonal thyroid weights suggest that thyroid activity is greater during the productive than the non-productive part of the year [Galpin, 1938], and from her figures it appears that thyroid weight is at a maximum (*i.e.* its activity is lowest) in the non-laying bird. On the other hand, Greenwood and Burns [1940] have demonstrated that the practically

continuous moult in the castrated bird is most intense at a time when thyroid secretion is regarded as approaching its maximum in the intact bird.

Some clue to the meaning of these paradoxical observations may be found if the possibility is considered that the conditions essential to the onset of the moult are not necessarily the same as those which obtain once it is under way. It may be, as the work of Giacomini and Zavadowsky suggest, that the impetus to plumage change is derived from a brief release of an increased thyroid secretion, or, what would have the same effect, the normal secretion may become "available" for this purpose through the cessation or slackening of the inhibiting action of the gonad. The first supposition might be tenable in regard to Hill and Parkes' experimental birds, or to hens, where sexual activity ceases abruptly in the autumn, but the slower moult of cocks and capons, combined with the continuation of sperm production at a reduced rate in the former [Greenwood, 1936], suggest that the second postulation is more probable. Such a switch-over in the demands made on the hormone from one part of the organism to another in no way disagrees with the deduction from the study of gland weights, and from the present experiments, that it is a season of low activity for the thyroid gland. Indeed, it could conceivably have resulted from the lowering of the hormone-level below that adequate for gonad function, though the more obvious explanation of the cessation of gonad activity would be a deficiency of gonadotrophins. In this connection it has further to be considered whether lowered pituitary function is concerned solely with its gonadotrophic activities, or whether its thyreotrophic action is also involved, and so responsible for the change in level of thyroid secretion.

Whatever may be the exact relationship between gonad, pituitary, and thyroid such considerations suggest that in the fowl the thyreotrophic action of the pituitary has an important reciprocal counterpart in the influence of the thyroid on that gland, and through it on the functioning of the gonads.

Summary.

1. Extracts of Horse, Sheep, and Pig Pituitary, Pregnant Mare's Serum and Chorionic Gonadotrophin were administered to hens prior to and during the moulting period. Dried thyroid powder was fed during the non-productive phase.

2. Injections of P.M.S. to laying hens resulted in cessation of egg production within 5 days, and the onset of a heavy moult. The combs increased in size during treatment. Apart from a slight increase in comb size in the group receiving Pig Pituitary extract, no reaction to the other gonadotrophins could be detected at the dosage-level employed.

3. In non-laying hens P.M.S. had little effect; Pig Pituitary appeared to cause a temporary reactivation of the reproductive organs, but in no case did ovulation occur. The other gonadotrophins were again inactive.

4. Consistent symptoms of ovarian stimulation resulted from the administration of thyroid, and a number of birds resumed egg production following this treatment. Variations in the degree of response obtained appeared to be related to the media in which this hormone powder was fed.

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A QUANTITATIVE METHOD FOR THE PRODUCTION OF EXPERIMENTAL TRAUMATIC SHOCK WITHOUT HÆMORRHAGE IN UNANÆSTHETIZED ANIMALS.[1]

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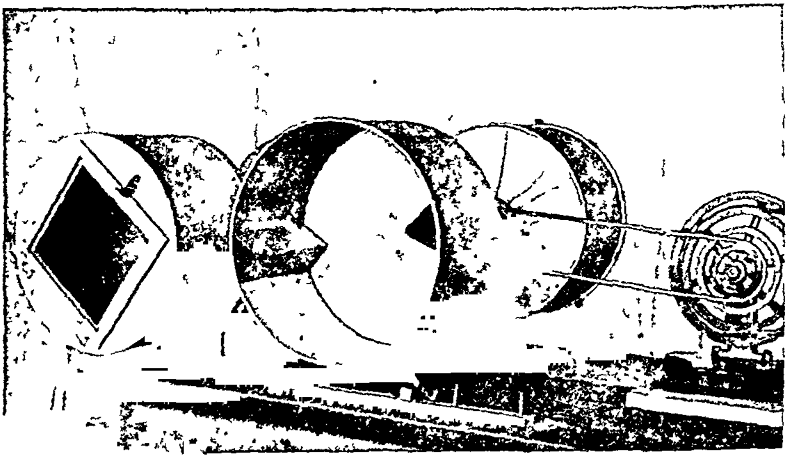
IN a previous publication the effects of histamine on rats were studied, with special reference to shock and the relationship of the adrenal glands [Noble and Collip, 1941]. Histamine was selected as a pure chemical substance of possible physiological significance with which to induce a picture resembling shock. With this method quantitative results could be obtained without hæmorrhage, infection, or anæsthesia as complicating factors. At this time a number of ways of producing direct trauma to the tissues of the rat were investigated, but without consistent or satisfactory results. These included prolonged ischæmia of one or two extremities; crushing of skin, muscles, or intestine; rapid freezing of limbs, skin and muscle, or intestine; scalding of skin and muscle. Moon [1938] has reviewed most of the experimental methods used to produce direct traumatic shock, but all may be included in the following criticisms; they are associated with at least one of the following—hæmorrhage, infection or anæsthesia—and the degree of trauma cannot be measured in a strictly quantitative fashion for duplicate experiments. Also, with the methods published it has not been practical to study the effects of trauma applied daily for prolonged periods. It was thought, therefore, that some other method of producing shock, which would overcome these difficulties, was desirable in order to conduct a comprehensive study on the ætiology of shock and to test various therapeutic procedures. In the present paper the results are described which have been obtained using a method in which trauma was applied in a graded, controlled manner, following which a typical picture of shock developed, uncomplicated by infection or hæmorrhage. Unanæsthetized rats and guinea-pigs have been studied extensively, but the method could be modified for other species.

[1] The findings recorded in this paper are a continuation of those contained in previous reports to the National Research Council of Canada, dating from 4th January 1941.

The criteria which have been described to identify shock or to indicate the degree of shock are extremely numerous and frequently open to criticism. Changes in blood pressure have often been recorded, but these are influenced by anæsthesia and hæmorrhage. Blood-volume measurements are inaccurate through the loss of dye into the tissues. Hæmoglobin values and red-cell counts are seriously affected by associated hæmorrhage. Many of the chemical changes are inconstant and are probably secondary to other factors. Although some of the findings associated with shock have been recorded with the method to be described, the death or survival of the animal has been used in all cases and is emphasized as the most accurate indicator for experimental shock.

Method and Apparatus.

The underlying principle of the method is to traumatize the animal by placing it in a revolving circular drum in which are projections,



or bumps. These carry the animal up the side during a turn, and it is then dropped, to be picked up by the following projection. The distance the animal falls is such that obvious hæmorrhage or fracture of bones are not produced. The apparatus may be seen in the figure, and consists essentially of a pair, or pairs, of identical circular galvanized iron drums connected by a short externally fixed, central shaft running in ball-bearings. On the shaft are various-sized pulley wheels for the driving belt. The drums have an inside diameter of 15 inches and a depth of 8 inches. The projections are of a blunt triangular shape on cross-section, with a height of 2 inches and base of 3 inches. The outside of each drum is closed by a clip or hinged lid, part of which is covered by heavy cellophane or glass so that observations can be

made inside. It is essential that all parts and projections be closely fitted to prevent the rat's nails from catching in any cracks. The projections may screw on so that 1, 2, or 3 may be attached at equal distances apart. Each pair of drums is driven by a belt from a motor through a set of wheels or gears for adjustable speeds, and a suitable revolution counter is attached. By running animals in each drum at the same time, a strictly comparable control can be obtained for evaluating methods of treatment.

The animals used have been maintained under as standard conditions as possible. The rats were of a strain bred in the laboratory and fed on Purina Fox Chow. Animals which survived were killed at various intervals after trauma. Their organs were dissected, fixed in Bouin's solution, and weighed after partial dehydration in 70 per cent. alcohol. Blood was taken from the heart or tail for hæmoglobin determinations, which were estimated using an electrophotocolorimeter. In each case control values were obtained on the animal beforehand. It was found necessary to ligate the end of the tail after bleeding to prevent subsequent hæmorrhage during running in the drum. The room temperature has been kept as constant as possible without thermostatic control.

Results.

Standard Technique.—Many modifications in technique were employed before results which were considered satisfactory were obtained. The finally adopted technique, therefore, will be described first, but the early observations will also be summarized, since they may be informative in a repetition of this work. The rats which have been used weighed between 150 and 200 g. Male animals were selected as near 160 g. as possible, and females at 150 g. The young guinea-pigs were of 150 to 250 g. For comparative mortality figures to be obtained the weights should be as standardized as possible. To prevent the rats jumping over the bumps it was found necessary to tape the paws with adhesive plaster. The front paws were therefore held by an inch-wide strip of plaster about half an inch apart. The hind paws were similarly taped just before the animal was placed in the machine. The tape was removed immediately following the run, to prevent struggling. Such a procedure was not necessary for guinea-pigs. The most satisfactory drum-speed was found to be at 40 revolutions per minute, and this has been kept constant to within 1 or 2 turns. Two bumps, as described, have been used in each drum. In Table I. the mortality figures and the time of death after the start of the shock application may be seen for male and female unanæsthetized rats, and similarly for guinea-pigs.

It may be noted that the number of animals dying showed a curve in proportion to the number of revolutions. Female rats apparently

TABLE I.—MORTALITY IN RATS AND GUINEA-PIGS FOLLOWING GRADED DEGREES OF TRAUMA.

(Standard technique.)

Revolutions.	Males.			Females.		
	No. of animals.	Per cent. mortality.	Average time of death (min.).	No. of animals.	Per cent. mortality.	Average time of death (min.).
Rats—						
800	10	100	47	12	100	39
700	10	100	50	12	75	81
600	10	80	58	12	66	77
500	10	70	71	12	50	116
400	18	50	152	12	9	
300	37	8	204			
200	10	0				
Guinea-pigs—						
250	14	85	30			
225–300	..	..	..	10	100	18
225	10	100	37			
200	14	21	60	10	90	207
175	..	..	..	14	35	24
150	..	..	..	10	30	22
125	..	..	..	10	0	

were about 100 turns more resistant than male animals, although the curves were quite parallel. Guinea-pigs were much more susceptible than rats, but the spread in the mortality figures was much less. Males were slightly more resistant than females. Rats apparently give more regular results, and have been found preferable to guinea-pigs in most studies using this method. The time of death after shock increased as the percentage mortality and number of revolutions became reduced. In the case of normal rats death has only occasionally occurred later than 6 hours after trauma. In the time of death recorded in Table I. animals dying after 6 hours have not been included.

Effects of Variations in Method.—In initial experiments the drum-speed was set at 21 revolutions per minute and 3 bumps were used. Female rats only were employed. When unanæsthetized animals were run without having their paws taped they were found to give irregular results, since some would at first jump over the bumps until fatigued, and so protect themselves. These results are shown in Table II., and those for animals having their paws taped run under comparable conditions are also included. To prevent the rats jumping a number were shocked while under light nembutal *anæsthesia.*

(Abbott's Veterinary Nembutal was given subcutaneously in doses of from 19 to 26 mg. per kg. approximately 30 minutes before shocking.) With this modification the rats were more susceptible to shock when compared with those having their paws taped, as may be seen in Table II. This method had to be abandoned, however, because approximately half the rats showed intra-abdominal hæmorrhage due to tears in the spleen (and rarely in the liver). Such animals are not included in these results. Apparently the relaxation of the abdominal muscles allows an increased amount of direct trauma to the spleen, as rupture of this organ has only exceptionally been observed in unanæsthetized animals. For example, rats which were kept in a state of surgical anæsthesia under ether for 5 minutes and then allowed 30 minutes to recover did not exhibit abdominal hæmorrhage. These results are also included in Table II., and it may be noted that such a procedure did not increase the susceptibility to shock.

A few animals with legs taped were run at a drum-speed of 33 revolutions per minute with only 2 bumps. The effects were of a similar order to those with the previously described speed, and are recorded in Table II. A speed of 59 revolutions per minute with 2 bumps was also used for some experiments. The effects at this speed on female and male rats with paws taped are also included in the table. These animals also showed a sex difference in that the females were more resistant. Guinea-pigs were run under some of the conditions stated above. Those at 21 revolutions with 3 bumps were subjected to preliminary ether anæsthesia as described for the rats, while those at 59 revolutions with 2 bumps were normal (Table II.). From direct observations on the animals when the drums are revolving, and from other considerations, it was concluded that the speeds of 21 and 33 turns were too slow and 59 was too rapid. Similarly, 3 bumps were less satisfactory than 2. As previously mentioned, therefore, a speed of 40 with 2 bumps was adopted for subsequent experiments. It has also been noted that a more regular degree of trauma might be produced if the diameter of the drum was increased slightly from ½ to 1½ inches. Similarly, if larger rats or guinea-pigs were to be used the depth of the bump could profitably be increased by about ½ inch.

One experiment of a different type may be mentioned. Rats after having their paws taped were run in the machine and observations were made every 100 revolutions until the animals died. With a speed of 21 r.p.m. and 3 bumps the number of turns required to kill female rats varied in 6 animals from 600 to 1400. This type of procedure was therefore abandoned.

Hæmoconcentration and Body Temperature.—Hæmoglobin determinations have been made on a number of rats subjected to trauma by this method, and in some cases rectal temperatures have also been recorded. In general it may be stated that the hæmoglobin values invariably

TABLE II.—MORTALITY IN RATS AND GUINEA-PIGS FOLLOWING GRADED DEGREES OF TRAUMA.

(Variations in technique.)

Conditions.	Revolutions.	No. of rats.	Per cent. mortality.
Speed—21 revs. per minute—3 bumps—female rats—			
Unanæsthetized—paws untaped	700–1000	15	93
	500–600	5	80
Unanæsthetized—paws taped	800	6	83
Nembutal anæsthesia	750	8	100
	500–600	10	10
Preliminary ether anæsthesia	750–800	12	75
Speed—33 revs. per minute—2 bumps—female rats—			
Unanæsthetized—paws taped	900–1000	4	100
	800	6	83
Speed—59 revs. per minute—2 bumps—female rats—			
Unanæsthetized—paws taped	700	11	100
	600	10	40
	500	4	0
Speed—59 revs. per minute—2 bumps—male rats—			
Unanæsthetized—paws taped	700	3	100
	600	6	83
	500	6	17
Speed—21 revs. per minute—3 bumps—female guinea-pigs—			
Unanæsthetized	200–250	8	100
	150–175	4	0
Speed—59 revs. per minute—2 bumps—female guinea-pigs—			
Preliminary ether anæsthesia	250–300	6	100
	200	4	25
	150	4	0
Speed—59 revs. per minute—2 bumps—male guinea-pigs—			
Preliminary ether anæsthesia	300	4	75
	225	4	0

increase, indicating hæmoconcentration. The percentage increase apparently depends on the severity and the time following trauma. A number of facts have been established and may be summarized.

In rats subjected to a degree of shock always rapidly fatal a moderate hæmoconcentration occurs. In 8 animals subjected to 800 turns with the standard technique, and which lived only some 5–15 minutes after removal from the machine, the hæmoglobin averaged 126 per cent. of the normal control values for the animals. Since this degree of concentration is quite compatible with life, the immediate cause of death was not due to hæmoconcentration *per se.* In animals which survive for longer periods after moderately severe trauma it has been found that, immediately after, the hæmoglobin values are increased from 5 to 15 per cent. Concentration may increase for 3 hours, and in some cases remains present for 5 hours or longer. With recovery the hæmoglobin values return rapidly to normal, and usually actual hæmodilution (values 80 to 95 per cent.) is observed. Hæmoconcentration in some cases may be nearly maximal, as values of a 50 per cent. increase have been observed. When individual values are considered a great deal of variation is found, depending on the animal and the degree of shock to which the animal is subjected. In most cases, once the hæmoconcentration starts to diminish, although this may occur slowly, the chances of the animal's survival are good. However, if the hæmoglobin increases as high as 130 per cent., or over, in nearly all cases death ensues. The degree and duration of the hæmoconcentration therefore serves some value as an approximate indication of whether the animal will eventually survive.

Immediately following trauma the rectal temperature of rats and guinea-pigs may be raised slightly, but after a few minutes it falls rapidly. Temperatures below 95° F. are frequently found, and in general these are encountered at the same time as severe hæmoconcentration. Although the temperature may drop as much as 7° in ½ to 1 hour, its fluctuation has been found to be less reliable than changes in hæmoconcentration as an indication of the severity of shock. The rectal temperature is undoubtedly markedly influenced by local blood-flow so that the alterations may be excessive if compared with temperature in other parts.

Picture presented by Shocked Animals.—Following the removal of the animals from the drums, they are seen to be quiet, appear ill, but are not unconscious, and if startled will readily respond by moving about. Their condition apparently becomes progressively worse for some hours. Respiration is usually increased in rate. In some cases blood may be seen around the margin of the mouth and nose, but this is slight, and free hæmorrhage has never been noted. Occasionally the teeth, especially the upper incisors, may be broken or knocked out. (In such cases the animals are given a powdered form of Purina to eat and are not considered in studies relating to weight disturbance.) If death follows rapidly the animal becomes moribund, respiration is slowed, and the heart-beat is irregular on palpation. Unconsciousness

is present for a short time before death but convulsions are rarely seen. Animals which are not so acutely ill gradually become quiet, lie prostrate, and drink water at frequent intervals. At an early stage severe diarrhœa is consistently observed. In guinea-pigs the changes are more acute, and most animals appear listless and quiet, with ruffled fur, until death or recovery.

At post-mortem, or if the animals be killed, the findings are typical, but vary in intensity, depending on the degree of shock and the time of death after trauma. In rats a certain amount of bruising may be seen in some animals, especially over the skull and paws. The muscles in general appear redder in colour and more vascular than normal. This is especially noticeable in the anterior abdominal muscles. Free hæmorrhage is present in less than 1 per cent. of cases and is found in the subcutaneous tissues over bony prominences, occasionally in abdominal or retroperitoneal muscles. In such cases the animals are discarded in the tabulation of results. Hæmorrhage into the skull or brain practically never occurs, although some increased vascularity may be present and the venous sinuses are prominent. The most striking changes are found in an animal some hours after shock, and are seen in the abdomen. These consist of extreme vascular dilatation, engorgement and congestion of the mesentery and bowel. The distal two-thirds of the small bowel frequently becomes very engorged and deep purple in colour. The cæcum similarly is nearly always affected, and the dilated vessels stand out clearly against the bluish-coloured muscle. Venous congestion of the rectum, duodenum and stomach may be present, but usually to a lesser degree. The contents of the bowel rapidly become fluid, and areas of congested mucosa of the small and large bowel desquamate into the lumen. The associated diarrhœa may be blood-stained. Acute hæmorrhagic erosions frequently occur in the stomach, ileum, and cæcum. The kidneys may appear congested and the urine frequently contains blood. The spleen is usually somewhat enlarged and congested from the trauma, but rupture or tears are infrequent in unanæsthetized animals, as previously mentioned. Small subcapsular hæmorrhage, however, may occur. The liver frequently shows contusion and engorgement, with hæmatoma, along the free margin, especially the anterior edge. The adrenal glands may show small hæmorrhages on their surface. In the thorax the changes are less striking. The heart may appear normal, but on cutting it only a small amount of dark-coloured thick blood escapes. The lungs usually are normal or slightly congested, but in some cases congestion may be more marked, with small petechial hæmorrhages present. Lung œdema is not usually encountered. Small hæmorrhages may occur into the thymus gland. It has been noted that in animals dying rapidly after severe trauma many of the changes described have not developed, or are present only to a slight extent. When the

typical picture is presented the animal usually has survived at least half an hour after being subjected to trauma.

When the animals recover but are killed after 24 or 48 hours a much more normal picture is presented, as healing is apparently rapid. In rats the intestinal congestion has disappeared and the bowel is usually contracted and empty. The cæcum shows the most consistent change and may be seen as large, subacute mucosal ulcerations. The stomach mucosa may show similar erosions. The spleen is contracted and occasionally shows evidence of previous subcapsular hæmorrhage (Table III.). The liver shows greyish healing scar areas. Very

TABLE III.—EFFECTS ON ORGAN WEIGHTS AFTER TRAUMA IN RATS.

No. of Revolutions.	Acute death.				Killed 24 hours.				Killed 48 hours.			
	No. of rats.	Spleen, mg.	Adrenals, mg.	Thymus, mg.	No. of rats.	Spleen, mg.	Adrenals, mg.	Thymus, mg.	No. of rats.	Spleen, mg.	Adrenals, mg.	Thymus, mg.
(Group A) 800	12	1103	48·4	188								
700	9	1163	43·7	166	1	680	52·0	195	2	685	53·5	125
600	8	1190	41·1	189	2	655	56·5	172	2	960	44·5	200
500	6	1081	49·3	184	6	773	53·8	174				
400	1	1310	42·0	255	5	616	45·4	157	6	696	41·5	159
Total .	36	1139	45·3	183	14	744	51·0	169	10	747	45·5	162
(Group B) *Sex.*												
Females .		1227 (27)	37 (29)	238 (34)	6	565	43	205	9	777	32	209
Males .		1543 (6)	24 (10)	308 (10)	3	1160	30	245				

occasionally other changes are found—the kidneys may be pale and yellow, clear fluid may be present in the peritoneal cavity, slight retroperitoneal œdema may be seen, especially near the adrenal glands. The thymus gland may show some degree of involution and the adrenal glands may be slightly enlarged. The weights of the spleen, adrenal and thymus glands of female rats subjected to various degrees of trauma and killed at various intervals are shown in Table III. These animals were taken from the series recorded in Table I.

Group B shown in the table tabulates the organ-weights for some of the animals which were used with modifications in technique, as noted in Table II. All these results have been averaged, but only animals subjected to a degree of trauma producing a 50 per cent. mortality have been included. The females were of a smaller size than regularly used and averaged 135 g. in body-weight.

In guinea-pigs the same general picture is presented as in rats. However, the occurrence of subcutaneous intramuscular and even intracranial hæmorrhage is much more frequent, and necessitates the discarding of a number of animals. The large bowel shows the most consistent and most marked changes. The lungs appear to show more congestion and œdema than do those of rats, and this may result in an increase in weight up to 20 per cent. Animals which recover appear normal in every respect, and no cases have been recorded where residual paralysis or other symptoms were seen, which might indicate damage to the nervous system. Similarly, no bony fractures have been observed. A loss in body-weight of 3 to 10 g. may occur in the first 24 hours after trauma, but this is temporary and the original weight is usually regained in 2 to 3 days. Histological examination of the various organs has only disclosed changes which were anticipated from the gross appearance and were similar to those reported by other workers in the pathology of shock.

Discussion.

The method which has been described to produce traumatic shock has been found to be more practical and to yield more satisfactory results than any other investigated in this laboratory. Since the *animals may be subjected to graded degrees of trauma it is possible to obtain strictly quantitative results. With this method* the complications of hæmorrhage, infection and anæsthesia, usually associated with experimentally produced shock, are absent, or reduced to a minimum. In rats dying after this procedure the symptoms and changes found are described as typical for shock [Moon, 1938]. The venous congestion and engorgement with cyanosis of the intestine, associated with severe hæmoconcentration, is extremely marked. In cases where recovery occurs no evidence of any unexpected lesion has been found, as might be expected if injury to the brain or nervous system was produced. When rats were killed at 24 and 48 hours after shock the intestine showed healing areas of ulceration and the spleen was contracted. From the weights recorded only slight adrenal enlargement was present at 24 hours and the thymus was slightly affected. Adrenal hypertrophy and thymus involution have been described by Selye [1936–1937] as typical changes indicative of the alarm reaction. Apparently the method of producing trauma described is associated with the development of only the mildest degree of alarm reaction.

The time of death after shock in rats and guinea-pigs appears to be rapid, when compared with the findings for other methods of producing shock and in clinical cases. Because of this difference it appeared questionable whether the method described for applying trauma resulted in death from shock or from some other cause, such

as the trauma itself. This point has been investigated as extensively as possible and, as has been pointed out, no evidence of any definite additional factor which might be the cause of death has been found. From the type of trauma which is used it might be suspected that damage to the nervous system could be responsible for the rapid death encountered. Some evidence contrary to this suggestion, however, may be mentioned. In a number of animals anæsthetized with nembutal an attempt to protect the spleen was made by taping the abdomen with adhesive. Of 8 animals only 2 died, whereas controls showed 100 per cent. mortality. In such cases the head and chest were quite unprotected. Other results, which will be published shortly, may also be mentioned. Adrenalectomized rats are much more susceptible to this form of trauma than are normals, but they may be protected by suitable injection therapy. In general it may be stated that all the evidence at present points to death being directly related to the amount of trauma applied, and a picture which is usually attributed to shock may be shown by the traumatized animal. It would appear that the rat has a considerable degree of resistance to shock and that a rapid return to normal occurs following trauma. With the present method there is no permanent lesion or laceration of tissue, so that trauma is really limited to the time in the machine. Therefore, it would appear logical that death should follow shortly after the trauma or not at all once recovery processes set in. A comparison of the time of death after this type of trauma and that following permanent damage produced by pounding, crushing methods might be expected therefore to yield divergent results in the order of those reported.

The findings which have been obtained up to the present are most readily interpreted by the hypothesis which has been advanced by many that a toxic substance is produced in the tissues due to trauma. If this is the case it would explain why some animals may die immediately following trauma and why, once this type of trauma ceases, the production of a toxic substance stops. In the animals where death follows almost at once the typical shock picture may be only partially developed and hæmoconcentration present only to a slight degree. These features must therefore be secondary to the liberation of the toxic substance. It would appear that if enough of the toxic substance enters the blood stream it produces death rapidly, possibly by affecting the heart or brain. In an animal receiving trauma for a short period the peripheral circulation is probably relatively good and therefore the toxic substance may be rapidly removed from the damaged tissue to the blood stream. On the other hand, in an animal subjected to trauma over a prolonged period the circulation may be poor and the toxic product released slowly causing death at a later interval. Somewhat similar conditions may influence the concentration of the blood. In

animals dying rapidly little hæmoconcentration may be found. However, in the presence of traumatized tissues, over a longer period, marked loss of plasma from the blood may occur. It would appear that if the toxic substance produced remains in the tissues, as when the peripheral circulation is poor, the loss of fluid locally is marked. In such cases the death of the animal is related to a direct toxic effect plus the result of hæmoconcentration. Further, when hæmoconcentration is advanced the oxygen supply to the peripheral tissues is deficient. Such anoxæmia may lead to a further local increase in the production of the toxic substance. Such an argument would correlate the type of shock caused by methods employing temporary occlusion of the blood supply to some part of the body with that following direct trauma, since the end result would be the liberation of the same toxic product. The above theory for the ætiology of the development of shock, which has been evolved from the experiments described and others to be published, is similar in some ways to that of Moon. However, in the theory outlined the peripheral tissues are suggested as the site where some fundamental chemical alteration occurs following trauma or ischæmia, and this results in the production of a toxic substance. This substance may act locally, resulting in the attraction of plasma with hæmoconcentration (which in turn causes secondary general ischæmia and additional liberation of the toxic material), or systemically, possibly affecting the heart and brain, and even producing rapid death. Whether the substance acts locally or enters the systemic system may in itself be determined or influenced by the state of the cardiovascular system and peripheral circulation. In such a plan the capillaries would have a passive rôle and be affected secondarily rather than be the primary site of the damage responsible for the development of the shock picture, as outlined by Moon.

If this explanation is correct the types of shock, as seen both experimentally and clinically, might be expected to show marked variations. At the one extreme would be cases where death followed trauma very rapidly, and at the other those which ran a protracted course and in which increasing hæmoconcentration was a prominent finding. Therapeutic procedures which tend to alleviate increased blood concentration might be expected, therefore, to be beneficial in the latter type of case only.

Summary.

A method has been described in detail by which rats and guinea-pigs were subjected to graded degrees of trauma. Following such a procedure mortality curves were obtained indicating that strictly quantitative results could be produced. Animals subjected to this type of trauma develop hæmoconcentration and the pathological changes ascribed to the condition of shock. The complications of

hæmorrhage, infection and anæsthesia are not associated with the shock so produced.

Some theories for the ætiology of shock are discussed, and from the experimental results obtained a modification of the toxic substance theory is suggested.

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ADRENAL AND OTHER FACTORS AFFECTING EXPERIMENTAL TRAUMATIC SHOCK IN THE RAT.

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In a previous paper a quantitative method for producing traumatic shock has been described [Noble and Collip, 1941 *a*]. In rats subjected to this type of shock the number of animals dying was shown to be directly related to the amount of trauma. This appeared, therefore, to offer a sensitive means by which to determine what influence the adrenal glands and other factors might exert on shock. In the present paper the observations have been continued, using adrenalectomized and hypophysectomized rats. Adrenal cortical extracts have been tested extensively on normal and adrenalectomized animals. The effects of ether anæsthesia and of hæmorrhage on traumatic shock have also been studied.

Methods.

The apparatus and method of applying trauma was the same standard technique as previously described, and the amount of trauma was proportional to the number of revolutions recorded. The rats were of a hooded strain maintained in the laboratory, and were selected when possible so that they weighed from 150 to 170 g. at the time when they were shocked. They were fed on Purina Fox Chow. All adrenalectomized animals were given 0·9 per cent. NaCl to drink instead of water irrespective of whether they received other treatment or not, and showed an increasing body-weight. If these animals survived shocking they were placed on water after a few days, and unless a loss of weight and death followed within two weeks the animals were discarded and considered to be incompletely adrenalectomized. Hæmoglobin determinations were made, using an electrophotocolorimeter, on 0·05 c.c. of blood drawn from the tail. The solutions were cleared by the addition of ammonia. Desoxycorticosterone acetate was administered as large single pellets (60 to 100 mg.) made by com-

[1] The findings recorded in this paper are a continuation of those contained in previous reports to the National Research Council of Canada, dated 4th January 1941.

pression without any binding agent. The adrenal cortical extracts used were generously supplied by Upjohn Company, containing 50 dog units per c.c., and Connaught Laboratory, containing 30 dog units per c.c., and an extract kindly sent from Dr. E. C. Kendall. The corticotrophic extracts of the pituitary were made in a similar fashion to those described [Noble and Collip, 1941 *b*], and were augmented in the same manner.

Results.

Relationship of Adrenals and Shock.

Adrenalectomy.—In the initial experiments adrenalectomized rats of both sexes maintained on oral saline for 4 to 15 days were shocked and the mortality figures compared with normal rats. These results are recorded in Table I. The data for normal animals is taken from a previous paper. It may be seen that removal of the adrenals rendered the animals much more susceptible to this type of shock. Female rats, both normal and adrenalectomized, were slightly more resistant than males. Groups of animals have been tested for 4 to 15 days after adrenalectomy, but without any significant difference being found in the mortality figures.

Adrenalectomy and Cortin Treatment.—Immediately following the removal of the adrenals the rats were treated with cortin. Subcutaneous injections were given twice daily for 4 or 5 days. On the day of the test 5 hourly injections were given and the animal shocked 15 minutes after the last injection. The three preparations of cortin gave practically identical results, so these have been averaged. The doses used were: Upjohn—$\frac{1}{8}$ c.c. twice daily, $\frac{1}{4}$ c.c. hourly; Connaught—$\frac{1}{4}$ c.c. twice daily, $\frac{1}{2}$ c.c. hourly; Kendall—$\frac{1}{20}$ c.c. twice daily, $\frac{1}{4}$ c.c. hourly. From Table I. it may be seen that cortin treatment completely restored the resistance of the adrenalectomized rats and that the figures obtained were actually slightly above normal.

Adrenalectomy and Desoxycorticosterone Acetate Treatment.—The pellets of desoxycorticosterone were implanted into the subcutaneous tissues at the time when the adrenal glands were removed. The animals were shocked from 6 to 12 days later. In one experiment the pellets were weighed at the time of insertion and after removal from the animal. The average loss in weight of 6 pellets was 18·3 mg. in 26 days. From the results in Table I. it may be seen that this form of treatment raised the resistance of the rats to above that of adrenalectomized ones and practically to normal.

Normals and Cortin Treatment.—The treatment with cortin as described for adrenalectomized rats was repeated exactly on normal ones. In two experiments water was given to drink and in one 0·9 per cent. saline, but without markedly influencing the results. From

Table I. it may be noted that cortin definitely raised the resistance of the normal rat. This was clearly seen at 600 turns, but only a few animals were able to survive 800 turns.

Normals and Desoxycorticosterone Treatment.—The administration of pellets was similar to that described for the adrenalectomized rats. It may be seen that this form of treatment was less effective than cortin but raised the resistance above normal. Both the adrenalectomized and normal animals appeared to be affected to about the same degree by this therapy.

Normals and Corticotrophic Hormone Treatment.—From the results obtained on early experiments before the conditions of the shock method were standardized it appeared that corticotrophic extracts exerted a beneficial effect on shock. For example, in one experiment with 8 hypophysectomized rats from 550 to 750 turns resulted in a 50 per cent. mortality. In 8 similar animals, but treated with a

TABLE I.—PER CENT. MORTALITY AFTER VARIOUS AMOUNTS OF TRAUMA IN NORMAL AND TREATED RATS.

(Number of animals shown in brackets.)

Condition and Treatment.	Number of revolutions.						
	800.	700.	600.	500.	400.	300.	200.
MALES.							
Normal	100 (10)	100 (10)	80 (10)	70 (10)	50 (18)	8 (37)	0 (10)
Normals—cortin . .	87 (8)	..	14 (7)				
Normals (and oral NaCl) .	60 (5)						
Normals—desoxycorticosterone.	77 (9)	..	44 (9)				
Normals—corticotrophin A	72 (11)						
Normals—inactive control extract C.	100 (4)						
Normals—corticotrophin B	..	60 (5)					
Adrenalectomized . .	..	..	..	..	77 (9)	68 (25)	6 (16)
Adrenalectomized—cortin	..	46 (13)	..	21 (19)			
Adrenalectomized—desoxycorticosterone.	..	..	..	83 (6)	43 (7)	0 (7)	
FEMALES.							
Normal	100 (12)	75 (12)	66 (12)	50 (12)	9 (12)		
Normals—corticotrophin B	..	75 (4)	..	0 (7)			
Adrenalectomized . .	..	..	..	87 (8)	66 (6)	0 (5)	0 (6)
Hypophysectomized .	87 (8)	..	90 (10)	..	44 (9)		

Average adrenal weights—Corticotrophic A—62 mg.
" " " — " B—males = 70 mg.
" " " — " B—females = 83 mg.
" " " —Inactive control C—males = 34 mg.

corticotrophic extract and subjected to 800 turns, no mortality resulted. The average adrenals weighed 13 mg. in the untreated group and 50·4 mg. in those treated. Using the standard method for producing shock the effects of corticotrophin on normal rats has been determined and the results are included in Table I. It may be seen that such treatment enabled the rats to resist shock to an appreciable degree.

Hypophysectomized Animals.—A number of rats were subjected to shock, from 13 to 20 days after removal of the pituitary gland. These results have also been included in Table I. The hypophysectomized animal was definitely more susceptible to shock than the normal, and appeared to react very similarly to the adrenalectomized.

Hæmoconcentration.—Since one of the typical findings after the application of trauma was hæmoconcentration, a series of observations have been made on the animals described above. Alterations in hæmoglobin have been taken as an indication of changes in blood-volume. In each case the animal was bled before shocking, and values obtained after were expressed as a percentage of the control. The actual amount of hæmoglobin is also included for the control value (calculated as for human blood). These results are shown in Table II.

TABLE II.—HÆMOGLOBIN VALUES IN NORMAL AND TREATED MALE RATS SURVIVING FIVE HOURS AFTER TRAUMA.

Condition and treatment.	No. of revolutions.	No. of rats.	Hb. g. /100 c.c.	Average hæmoglobin percentage.			
				Start.	1 hour.	3 hours.	5 hours.
Normals	300	8	12·77	100	107	105	99
,,	500	5	11·55	100	116	106	99
Adrenalectomized	200	4	11·98	100	106	106	102
Normals—cortin	600–800	8	11·87	100	107	115	111
Normals—desoxycorticosterone acetate.	600	7	11·44	100	111	104	104
Adrenalectomized—cortin.	500–700	7	11·22	100	117	112	111

In normal rats after 300 turns a slight degree of hæmoconcentration was present, but this was more marked after 500 turns. Increased values were present for 1 and 3 hours but had returned to normal 5 hours after shock. Individual variation in these and the other animals recorded was very great. An increase in hæmoglobin to *+29 per cent.* was recorded in one case, and following blood concentration a dilution usually occurred with resulting hæmoglobin values as low as 80 per cent. of normal. Untreated adrenalectomized rats

showed evidence of blood concentration even after 200 turns, and this was of slightly longer duration than that found in normals. After cortin treatment the blood was still found to concentrate and little dilution had taken place in 5 hours' time. This also occurred after treatment with desoxycorticosterone. The results recorded are difficult to compare, since the number of revolutions was so varied and the effects of treatment on resistance so different. It may be stated, however, that the methods of treatment employed did not prevent hæmoconcentration, although they may have modified it somewhat. It was also noted that normal animals consistently showed severe diarrhœa after shock, with apparent loss of fluid. Animals treated with cortin, however, developed relatively little diarrhœa.

Effect of Ether Anæsthesia and Hæmorrhage.—A number of different procedures were employed to determine the effects of hæmorrhage. The blood was removed rapidly by heart puncture either before or after shock. The amount bled was proportional to the body-weight, either 1 or 2 c.c. per 100 g. At autopsy any animals showing evidence of bleeding into the pericardium were discarded. Bleeding was performed under brief ether anæsthesia of less than 5 minutes' duration. Control experiments on the effects of ether alone were therefore conducted. In most cases the animals were divided into pairs and run at the same time in the paired drums of the shock machine. Rats of both sexes were used, and for comparative effects the sex difference should be noted. The results of these experiments may be seen in Table III.

It may be noted that preliminary ether anæsthesia or bleeding 30 minutes before shock did not apparently increase the susceptibility to shock. In fact, the bled animals appeared slightly more resistant. When blood was removed immediately, 10, 30, 60, or 120 minutes after shock there was no evidence that this increased the mortality. The number of animals surviving again seemed higher than normal. The same effect apparently was produced by the ether anæsthesia alone.

Effect of Evisceration.—The extensive changes which were observed in the intestine after shock suggested that the eviscerate animal might prove of value in studies on the ætiology of shock. The intestine, from rectum to duodenum, was therefore tied off and removed under ether anæsthesia in a number of animals. The blood-supply to the stomach, liver, and spleen was carefully preserved. Such animals quickly recover from the operation and may be killed 12 hours later in good general condition. A number have been shocked 30 to 45 minutes after such an operation. These animals, even after a large number of turns, appear well and are unmistakably more resistant than normal rats to this type of shock. Death occurs eventually, but after a much longer interval than in normals. For example, 4 female rats were eviscerated and subjected to 800 turns. None of these died before

Table III.—Effects of Ether Anæsthesia and Hæmorrhage on Mortality after Shock.

Treatment.	Sex.	No. of revolutions.	No. of rats.	Per cent. mortality.	Ether controls not bled. No. of rats.	Ether controls not bled. Per cent. mortality.
	M	700	..	..	10	100
Bled 1·0 c.c./100 g. 30 minutes before.	M	500	10	80		
		400	8	25		
		300	9	0		
Bled 2·0 c.c./100 g. 30 minutes before.	M	400	8	37		
Bled 0·7–1·0 c.c./100 g. immediately after.	M	300	8	0		
Bled 1·0 c.c./100 g. 10 minutes after.	F	600	2	50	1	0
		500	4	25	4	0
		400	0	8	8	0
Bled 1·0 c.c./100 g. 30 minutes after.	F	500	2	0	2	50
Bled 1·0 c.c./100 g. 60 minutes after.	F	500	2	0	1	0
Bled 1·0 c.c./100 g. 120 minutes after.	F	500	2	0	1	0

5 hours and the average time of death was 8 hours later. This effect has also been demonstrated with guinea-pigs. Taping the abdomen with strips of adhesive also afforded some protection against the trauma. The degree of protection has been found to be roughly proportional to the amount of tape used. In one case 4 male animals were each taped with 2 strips of adhesive 1 inch wide and 12 inches long. Following 800 turns 3 of the animals died at an average time of 3 hours and 40 minutes later. When 4 strips of the same size were used, in a comparable experiment, only 1 out of 4 rats died.

Discussion.

The rôle of the adrenal glands in shock has become increasingly important because of the possible benefit of therapy using cortical extracts. At the present time a number of reports have indicated that cortin and corticosterone are beneficial in the treatment or prevention of shock [Selye, Dosne, Bassett and Whittaker, 1940; Weil, Rose and Browne, 1940; Selye and Dosne, 1940]. In the use of desoxycorticosterone, however, some divergent results have been obtained. The above workers found it of little value, whereas Perla, Freiman,

Sandberg and Greenberg [1940] reported beneficial effects. Swingle, Hays, Remington, Collings and Parkins [1941] have noted a difference in the response to treatment of adrenalectomized dogs depending on whether trauma was applied to muscle or to bowel. Desoxycorticosterone was apparently of value in the former type of trauma only.

In the experiments described the beneficial effects of cortical preparations were first confirmed using adrenalectomized animals. It may be noted that such rats, even though growth and health were maintained from drinking saline, were much more susceptible than normals to shock from trauma. Under the conditions described preliminary treatment with cortin very definitely raised the resistance of the animals, so that they were slightly more resistant than normal. The time and manner of treatment was believed to be important, as cortin injections if given only the day previous, or the same day, were not found to give as striking results, and a single or double injection was of little value. When adrenalectomized rats were pretreated with large doses of desoxycorticosterone acetate, administered as pellets, to procure gradual absorption a definite increase in resistance was noted. This, however, was not as great as that obtained from cortical extracts. It should be pointed out that in all the experiments recorded the effects obtained were on the prevention of shock by pretreatment, since no treatment was administered after trauma, except where pellets were used. A continuation of treatment after trauma might have resulted in greater protection, but this can only be settled by experiment.

Having evolved a form of pretreatment which would protect adrenalectomized rats, its action on normal animals was then determined. Cortin was found in this case slightly to raise the resistance against shock. This was most apparent with 600 turns, but was not of any great magnitude since 800 turns resulted in a high mortality. In 5 animals given saline to drink in addition the changes were of a similar order. Desoxycorticosterone treatment also proved of slight benefit. Stimulation of the animal's adrenals by corticotrophic extracts also resulted in a slight lowering of the mortality following shock. In general it may be stated that the method of shock employed is probably a sensitive means of determining the value of treatment. Although the beneficial effects described have been definite the amount of protection afforded has been disappointing. From a practical standpoint the relatively large doses employed have proved of only little value. It is possible that with continued treatment, or with a different spacing of doses, the results might have been more encouraging. At the present time it is believed that the form of treatment described would be of only slight practical value. It has been established that the method of producing shock is not a limiting factor in these experiments. Using the same method of trauma it has been possible to

obtain large numbers of rats which withstood 1000 revolutions without any mortality, as will be reported at a future date.

The studies on hæmoconcentration after shock and the effects of the forms of pretreatment described have yielded varied results. In a previous report [Noble and Collip, 1941 *c*] it was found that the hypophysectomized rat showed a much greater and more prolonged hæmoconcentration than did normals after the injection of histamine. Treatment with corticotrophic extracts not only raised the lowered resistance of the animals but prevented the changes in hæmoconcentration, so that a normal response was obtained. This effect was presumably through the adrenal glands. Following trauma, however, the adrenalectomized rats did not show particularly severe blood concentration. On the other hand, it was only possible to subject such animals to a small amount of trauma and have them survive. Comparison with the normal is therefore difficult, but it appeared that normal rats gave a slightly smaller effect even after a greater amount of trauma. Cortin and desoxycorticosterone pretreatment did not prevent hæmoconcentration in the rats, although the same number of turns to which they were subjected would probably have produced a higher degree of hæmoconcentration in normal animals. It is possible that the increased susceptibility of the adrenalectomized animal to shock might be related to the additional toxic effects of hæmoconcentration and that the value of cortin might be in preventing such blood concentration. The results at present do not appear to justify this conclusion, but a large series of animals in which the blood changes in those dying acutely could be included might be of interest. Cortical extracts, therefore, might prove of greater value in combating hæmoconcentration and might be found more effective in the treatment of the type of shock where blood concentration is the predominant feature. As previously mentioned [Noble and Collip, 1941 *a*], it is thought that the mortality resulting from the shock method described is primarily toxic in nature and that hæmoconcentration is a much less important secondary factor.

From the reported observations on shock, especially in humans, it would appear that hæmorrhage and anæsthesia are definite factors which affect the severity and chances of survival. The results recorded on rats, therefore, are difficult to explain. Apparently in nearly all cases brief ether anæsthesia and bleeding before or after trauma did not increase the mortality figures. The reason for this discrepancy with other workers is not known, but may depend on the degree of hæmorrhage or the type of shock (as mentioned above) which this method induces. Hæmorrhage might be expected to increase the anoxæmia in types of shock where hæmoconcentration was severe. On the other hand, preliminary hæmorrhage by inducing hæmodilution might actually be beneficial in shock. The time factor between

hæmorrhage and shock probably is of greater importance than generally noted.

When the present method of shock was first described reasons were recorded why it was believed that the rapid death of the animal was probably related to a toxic factor rather than to possible damage of the nervous system. The results described further tend to rule out any extraneous factor related to direct trauma, since this would be constant. By taping the abdomen it was found possible to protect the animal against shock, although the skull and chest were quite unprotected. This suggested that visceral trauma was an important factor in the cause of death. The results obtained that eviscerated rats were much less susceptible also supported this view. In view of the results of Swingle *et al.* [1941] it might be suggested that muscle and visceral trauma are both present in the type of shock described. This might explain why desoxycorticosterone was effective, since it prevents shock from muscle trauma. The effects of evisceration have also suggested a number of new experimental approaches to the problem of demonstrating and isolating the substance possibly produced during shock, and some of these are under investigation.

Summary.

Rats have been subjected to traumatic shock using the method previously described. It has been found that adrenalectomized rats are much more susceptible than normals to this type of trauma. Suitable pretreatment with cortical extract restored their resistance to slightly above normal, and desoxycorticosterone acetate had a less, but definite, beneficial action. The same method of pretreatment was used on normal rats. In this case cortin and desoxycorticosterone caused a slight increase in resistance. Corticotrophic extracts of the pituitary were also effective to about the same extent. From a practical viewpoint the effects of adrenal preparations on this type of shock have been disappointing.

The changes in blood concentration following trauma have been recorded and the effects of hæmorrhage and ether anæsthesia have been determined. These results are discussed in relation to the possible factors concerned in the ætiology of shock.

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BRONCHOMOTOR AND PULMONARY ARTERIAL PRESSURE RESPONSES TO NERVE STIMULATION.

By I. de Burgh Daly and Catherine O. Hebb.[1] From the Department of Physiology, University of Edinburgh.

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In isolated perfused lungs of the dog, stimulation of the stellate ganglion or thoracic vagosympathetic nerves (T.V.S.) has been shown to produce a rise of pulmonary arterial pressure (P.A.p.) in the absence of bronchomotor changes as measured by tidal air excursions [Daly and Euler, 1932]. These results support the view that these nerves contain vasomotor fibres to the lungs. They do not, however, militate against bronchomotor effects having some influence on the pulmonary vascular resistance; they only show that the resistance changes due to nerve excitation can occur independently of bronchial constriction. Recently we have had the opportunity of observing the effects of pulmonary nerve stimulation in the perfused whole animal under negative pressure ventilation. In many experiments strong stimuli caused complete bronchoconstriction which, among other effects, undoubtedly caused passive resistance changes in the pulmonary vascular bed reflected as alterations of P.A.p. In such experiments the activity of the pulmonary nerves progressively diminishes, and therefore the earlier in the experiment the tests are made the better will be the responses to nerve stimulation. This applies both to bronchomotor and vasomotor effects, so it is to be expected that the most marked P.A.p. responses due to true vasomotor activity will always be associated with passive alterations of P.A.p. of bronchomotor origin. It is therefore of paramount importance to determine whether it is possible to distinguish between active and passive P.A.p. changes. The method adopted in the present investigation for perfusion of the whole animal is described in the succeeding paper [Daly, Elsden, Hebb, Ludány, and Petrovskaia, 1942]. In some of the perfused animals one lung had been removed at a previous operation. We have also performed experiments on isolated lungs perfused by way of the pulmonary artery with heparinised blood.

In our experiments the lungs, placed in an air-tight chamber, are perfused at a constant blood inflow and ventilated by rhythmic changes

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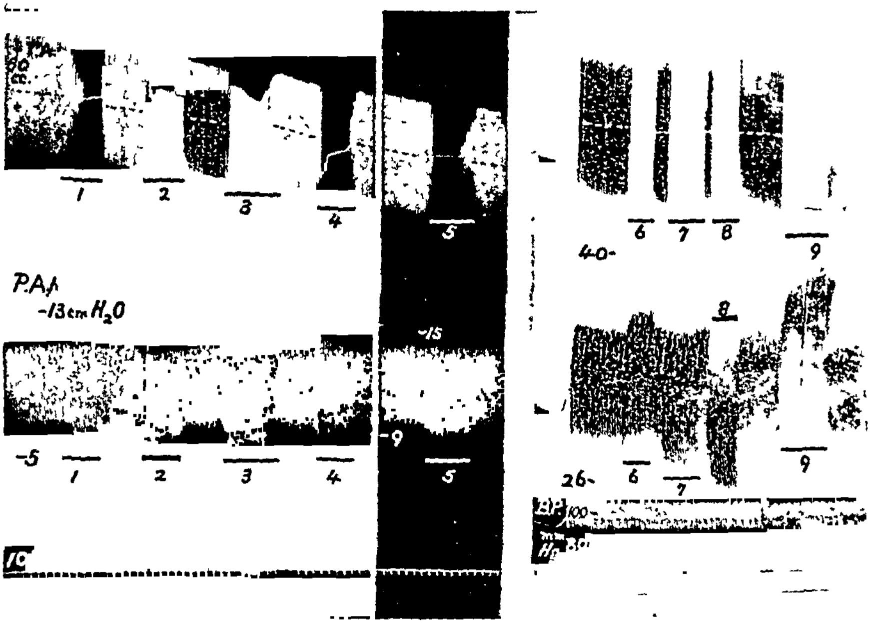
in extrapulmonary pressure. This is done by connecting a vacuum cleaner to the chamber, the resulting pressure fall in the chamber being nearly abolished sixteen times per minute by means of a valve which admits air to the chamber. The duration of the forces causing expansion of the lungs produced by the fall of extrapulmonary pressure is approximately equal to the duration of the elastic recoil of the lungs which causes almost complete collapse when air is suddenly admitted



(a) (b)

FIG. 1.—(a) Expt. 38/W.L.8. Dog, ♀, 5·7 kg. *Left lung removed at previous operation.* Isolated perfused lungs. Negative pressure ventilation (N.P.V.). At 1 and 5, gradual occlusion of trachea; at 2, 3, and 4, sudden occlusion of trachea. The release of the occlusion is gradual in all except 2. P.A.p. = pulmonary arterial pressure; T.A. = tidal air.

(b) Expt. 39/9. Dog, ♀, 6·6 kg. Perfusion of whole animal (P.W.A.). N.P.V. At 6, 7, 8, and 9, sudden and complete occlusion of trachea followed by sudden release.

The duration of occlusion in every case is shown by the horizontal white lines. Inspiration is downwards and expiration upwards in this and all T.A. tracings.

to the chamber through a wide-bore tube. This is shown by the fact that when the tracheal outlet is progressively obstructed by means of a screw-clip so as gradually to increase the resistance to air flow, the resultant static air volume of the lungs shows little or no change from the dynamic mean air volume (fig. 1, obs. 1). The static volume is defined as the quantity of air in the lungs after tracheal occlusion. Owing to there being differences in the visco-elastic properties of the lungs of the preparations used, gradual occlusion of the trachea did not always fix the lungs in the mid-respiratory position: in general 55–65

ıer cent. of the tidal air was entrapped in the lungs. It should be nentioned that the method of lung ventilation used automatically

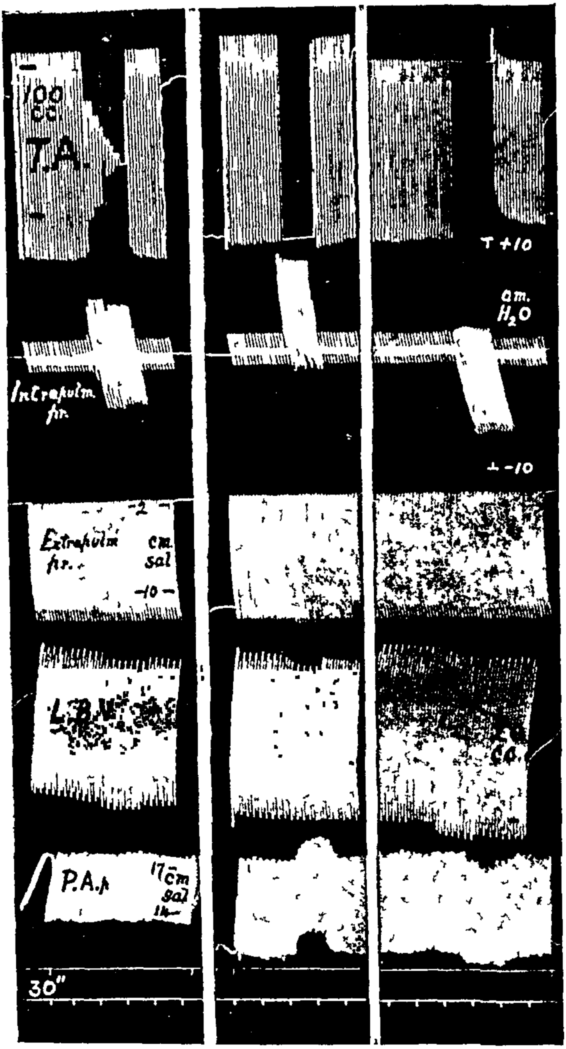


FIG. 2 —Dog, ♂, 10 3 kg Isolated perfused lungs Blood flow 600–700 c c /min. N P V. Showing similar effects to those in fig. 1, but intrapulmonary pressure and lung blood volume (L B.V) changes included Extrapulmonary pressure variations, −1·5 to −12 0 cm. H_2O. L B.V. increasing downwards

a=gradual occlusion of trachea, *b*=occlusion at full inspiration; *c*=occlusion at full expiration

results in the extrapulmonary pressure falling slightly more when the trachea is occluded than when air is allowed normally to enter the lungs [*cf*. Berry and Daly, 1931].

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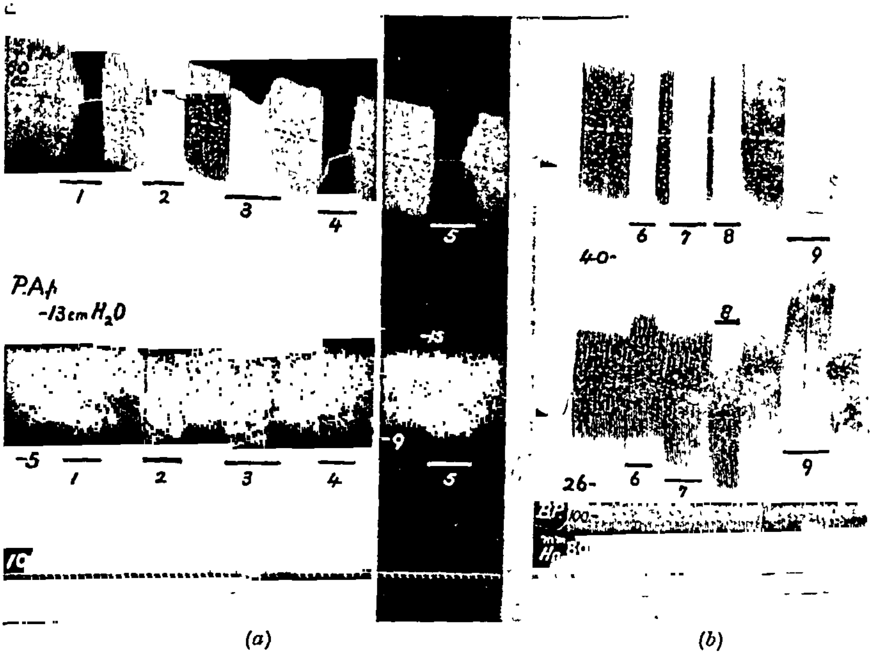

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that the change of P.A.p. which occurs when the trachea is suddenly occluded at full lung expansion will be determined not only by the subsequent intrapulmonary pressure rise, but by the absence of subsequent collapse of the lungs which normally increases the capillary

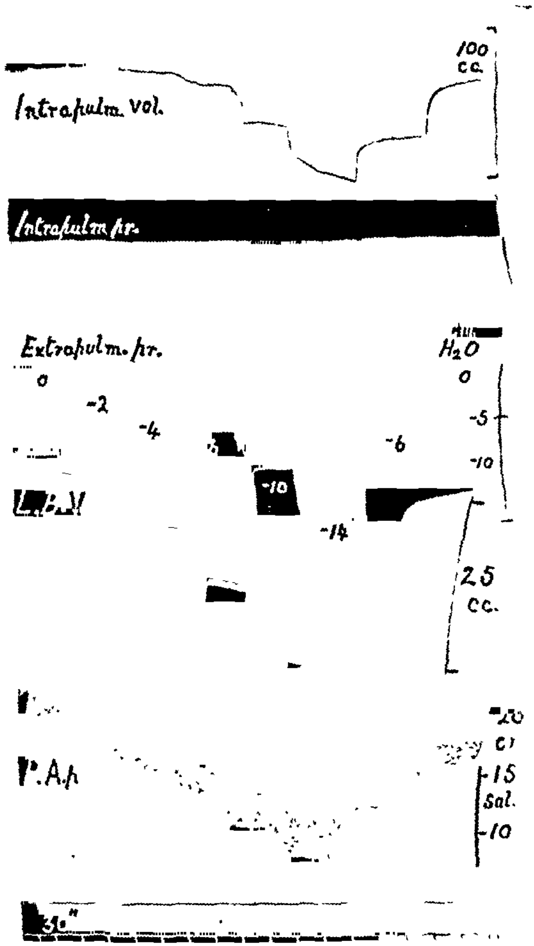

FIG. 3.—Expt. M.S.16. Dog, ♂, 10·3 kg. Isolated perfused lungs. N.P.V. Effect of alterations in extrapulmonary pressure on the P.A.p., lung blood volume and air volume. The extrapulmonary pressure was reduced in steps to −2, −4, −6, −10, −14 cm. H_2O and then raised to −6 and finally to 0 cm. H_2O. L.B.V. =lung blood volume, increasing downwards. The observations were made at the beginning of the experiment before ventilation, hence the small changes and the response lag of the intrapulmonary volume.

resistance and causes a P.A.p. rise. These two influences tend to balance one another, but it appears that the intrapulmonary pressure effect predominates so that the resultant effect is a P.A.p. rise. Reasoning on the same lines, the fall of P.A.p. due to the reduced mean intrapulmonary pressure caused by tracheal occlusion with the lungs in the

Mechanical Factors.—In order to throw light upon the mechanisms which determine the P.A.p. change during complete bronchoconstriction, we have attempted to simulate the intrapulmonary pressure changes which occur during vagal bronchoconstriction by suddenly occluding the tracheal outlet at different phases of the respiratory cycle. Sudden tracheal occlusion at the height of lung expansion leads, during the succeeding rise of extrapulmonary pressure, to a rise of mean intrapulmonary pressure due to the elastic forces of the lungs, which act to compress the air they are no longer able to expel. An intrapulmonary pressure rise causes, as is well known, a rise of P.A.p. due to compression of the capillaries. If, however, the trachea is occluded when the lungs are collapsed, no air can enter the lungs during the succeeding extrapulmonary pressure fall, with the result that the intrapulmonary pressure also falls causing a corresponding drop in the P.A.p. due to capillary dilatation (see fig. 1, obs. 4, 8, and 9; fig. 2). In all probability the intrapulmonary pressure changes described above are the main cause of the observed P.A.p. alterations during sudden tracheal occlusion. That other mechanisms also play some part we have no doubt.

There are three main mechanisms which govern the respiratory P.A.p. variations as well as the mean P.A.p. of lungs perfused at a constant blood inflow under "negative" pressure ventilation: (1) lung expansion *per se* which, owing to the internal architecture of the lungs, increases the calibre of the pulmonary capillaries. The resulting increase in blood capacity of the capillaries lowers the P.A.p. only whilst the lungs are *expanding*, and if this was the only factor, the P.A.p. would return to its initial value when the lungs had reached full expansion. The associated diminished capillary resistance, however, also lowers the P.A.p. during expansion and remains effective in keeping the P.A.p. down just as long as the lungs remain expanded. The P.A.p. is therefore partly determined by the degree of lung expansion (fig. 3). (2) The falling extrapulmonary pressure which increases the capacity of the relatively large blood-vessels lying on the surface of the lungs without producing a significant change in their resistance: this gives rise to a transient drop of P.A.p. only during the period of falling extrapulmonary pressure. (3) Changes in intrapulmonary pressure which are determined by the resistance to air entering or leaving the lungs. Normally a slight capillary dilatation producing a fall of P.A.p. occurs during normal lung expansion by virtue of the intrapulmonary pressure being reduced slightly below atmospheric pressure. Opposite effects take place during expiration. The greater the resistance to air flow and the greater the speed of ventilation, the more marked will these effects become.

These considerations are based upon the work of de Jager [1879], Dixon and Brodie [1903], Spee [1909], Romanoff [1911], and Daly [1930, 1938], as well as upon unpublished work. It should be noted

cycle. The slope of the curve CD, denoting the change of P.A.p. due to tracheal occlusion with extrapulmonary pressure variations of 0 to -12 mm. Hg is, as would be expected, steeper than that of AB taken with extrapulmonary pressure variations of 0 to -8 mm. Hg. The T.A. values before tracheal obstruction were 50 c.c. for curve AB and 100 c.c. for curve CD. The fact that the curve CD does not cross AB at the point corresponding to occlusion with 50 per cent. of the tidal air present in the lungs is probably due to the visco-elastic forces of the lung having changed as the result of altering the extrapulmonary pressure values.

These results suggest that when sudden and complete obstruction of the trachea occurs during ventilation of the lungs, the main mechanical factor which determines alterations of P.A.p. is the amount of air entrapped in the lungs. When the obstruction entraps more air than that contained in the lungs at the mid-respiratory position, the intrapulmonary pressure and P.A.p. rise, whereas when less air is entrapped than that held by the lungs in the mid-respiratory position the intrapulmonary pressure and P.A.p. fall. If no other factors are involved, it follows that the mean P.A.p. should not alter when complete and sudden obstruction takes place midway between expansion and collapse, provided also that in their duration the forces causing expansion and collapse are equal.

The effect of the degree of lung expansion on the capillary resistance in the absence of capacity effects can best be demonstrated by the application in steps of a steady extrapulmonary pressure to produce varying degrees of expansion (fig. 3).

The Relation between Bronchomotor Effects of Nervous Origin and the Pulmonary Arterial Pressure Response.—If we are so far correct in our interpretations, then complete and sudden bronchoconstriction produced by pulmonary nerve stimulation should cause similar qualitative changes in the P.A.p. as does tracheal occlusion, provided no direct effect on the pulmonary vessels is produced by the stimulation, and provided also that the elastic recoil of the lungs undergoes no change. We have examined the effects on the mean P.A.p. of some 70 stimulations of the caudal ends of the cervical vagosympathetic (C.V.S.), cervical vagus (C.V.), and cervical sympathetic (C.S.) nerves, all of which produced a concomitant bronchoconstriction, either complete or partial. When complete or partial bronchoconstriction has taken place, the percentage amount of tidal air retained in the lungs has never been greater than 55 per cent. and usually less than 50 per cent. (figs. 5 and 6). That is to say, the lungs have tended to collapse as bronchoconstriction supervened. Even when a sudden and complete bronchoconstriction has appeared likely to fix the lungs in the expanded position a final expulsion of air has apparently taken place (fig. 5, *a*, *d*). Since in these experiments the duration of the forces causing expansion

collapsed condition is greater than the fall of P.A.p. which would have occurred if the lungs had been allowed to expand.

No mention has been made of the effects on the P.A.p. of blood redistribution in the lungs which may be produced by tracheal obstruction during "negative" pressure ventilation. They are too complex to evaluate, but redistribution might cause changes in the volume

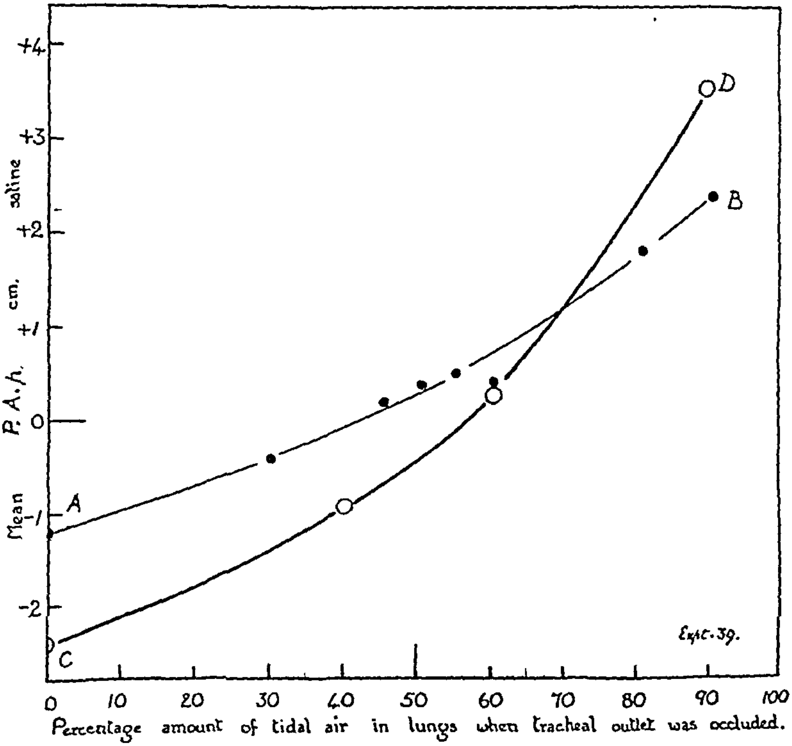

FIG. 4.—From same experiment (39/9) as fig. 1. Effect on the pulmonary arterial pressure of sudden and complete occlusion of the trachea at different phases of the respiratory cycle. AB = extrapulmonary pressure −0·5 to −8·0 cm. H_2O, tidal air before occlusion, 50 c.c. CD = extrapulmonary pressure −0·5 to −12·0 cm. H_2O, tidal air before occlusion, 120 c.c.

elasticity coefficients of the blood-vessels and so modify to some extent the relative effects on the P.A.p. of the factors enumerated above. For a similar reason the effect on the larger intrapulmonary blood-vessels of restricting lung expansion is not discussed. We believe, however, that since the capillaries form the greater part of the pulmonary resistance and contain at least twice as much blood as the rest of the lung vessels [Daly, 1938], they play the major part in determining P.A.p. changes brought about by mechanical influences.

The graph of fig. 4 shows the effect on the mean P.A.p. of sudden and complete tracheal occlusion at different phases of the respiratory

bronchoconstriction is sudden and complete, the intrapulmonary pressure change as well as the final position in which the lungs are fixed will govern the P.A.p. It follows that since under the conditions of our

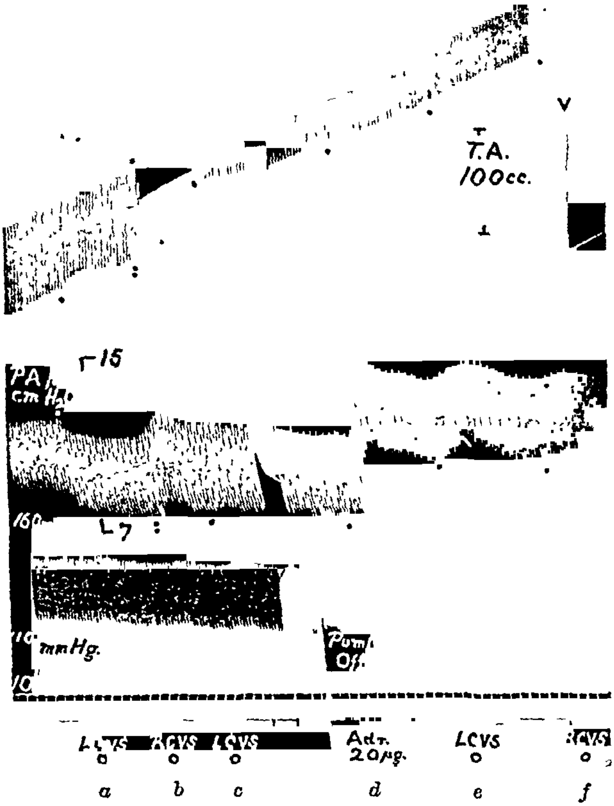

FIG. 6.—From same experiment (38/W.L.8) as fig. 1. Both C.V.S. nerves cut; eserine 2·0 mg. *Left lung removed at previous operation.*

a, *c*, and *e* = stimulation of caudal end of L.C.V.S., c.d. 0 cm.; *b* and *f* = stimulation of caudal end of R.C.V.S., c.d. 0 cm.

The systemic circulation pump was stopped shortly before *d*, when adrenaline 20 μg. was injected into the pulmonary arterial tubing. The T.A. tracing lever stuck at the top, and when moved downwards complete bronchoconstriction was found to have been produced by R.C.V.S. stimulation.

experiments the lungs always become immobilised towards the expiratory position during bronchoconstriction of vagal origin, the P.A.p. will rise if the bronchoconstriction is partial or, when complete, if its onset is slow enough to allow the intrapulmonary pressure to become equal to the atmospheric pressure. If, on the other hand, the bronchoconstriction is sudden and complete the P.A.p. will fall.

The volume of air entrapped in the lungs as a result of vagal bronchoconstriction will be, for any given extrapulmonary pressure variations, less than that entrapped by tracheal occlusion, owing to the former taking place

and collapse of the lung is approximately the same before nerve stimulation, we interpret these results as indicating that pulmonary nerve stimulation causes an increase in the viscance and/or elastance of the lungs, as Bayliss and Robertson [1939] found for vagal stimulation in the cat. Whether active contraction of the interstitial musculature of

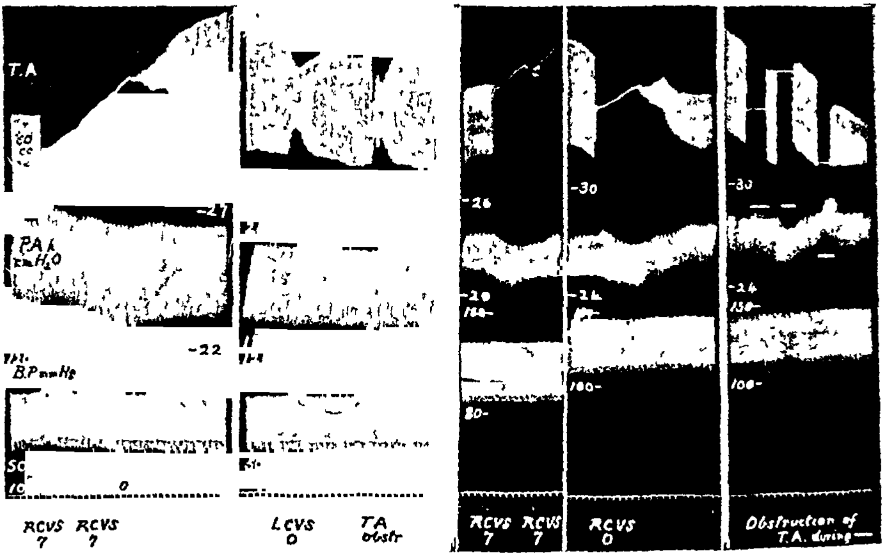

Fig. 5.—Expt. 31/2A. Dog, ♀, 4·7 kg. *Left lung removed at previous operation.* P.W.A., N.P.V. Both cervical vasosympathetic (C.V.S.) nerves cut. Eserine 2·0 mg. Caudal end of nerves stimulated in each case.

a = two stimulations of R.C.V.S., coil distance (c.d.) 7 cm.; *b* = stimulation of L.C.V.S., c.d. 0 cm., and tracheal occlusion followed by release; *c* = two stimulations of R.C.V.S., c.d. 7 cm.; *d* = stimulation of R.C.V.S., c.d. 0 cm.; *e* = three occlusions of trachea at different points in the respiratory cycle. The T.A. lever had reached its lowest possible level in last portion of tracing.

The rise of the T.A. tracing during complete bronchoconstriction in *a*, *c*, and *d* is due to the flow of oxygen into the closed circuit respiratory system. If this flow is perfectly balanced with the oxygen consumption of the animal, the T.A. tracing remains horizontal as at the beginning of *a*.

the lungs plays a part in the production of this phenomenon we are unable to state. If the lung "hindrance," a term suggested by Bayliss and Robertson to denote the total force developed in response to a unit deformation at unit rate, is increased during the development of complete bronchoconstriction, the effect upon the P.A.p. will be complex depending upon the rate of development of lung "hindrance" leading to complete bronchoconstriction. If the complete bronchoconstriction of vagal origin takes place slowly enough to allow the mean intrapulmonary pressure to become equal to atmospheric pressure, then the position in which the lungs are finally *immobilised* will be the main mechanical factor determining the P.A.p. change. If, however, the

slight bronchoconstriction during which the dynamic mean air volume alters little, therefore no passive effects on the P.A.p. would be expected

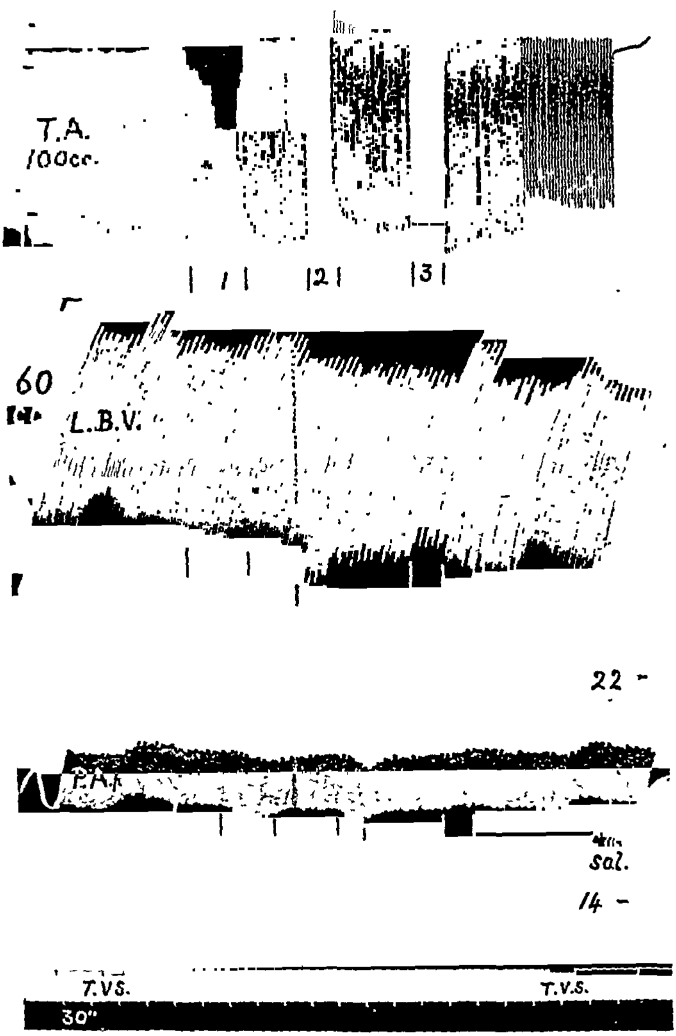

FIG. 7.—Expt. M.S.13. Dog, ♀. 15·0 kg. Isolated perfused lungs. N.P.V. Two stimulations of the thoracic vagosympathetic (T.V.S.) nerve, c.d. 0 cm., are shown at the beginning and end of the record.

1 = gradual tracheal occlusion followed by release. 2 = sudden and complete occlusion of trachea at height of expiration, and 3, at height of inspiration.

to take place. The second L.C.V.S. nerve stimulation, which is accompanied by a slight release of the complete bronchoconstriction produced by a previous stimulation of the R.C.V.S. nerve, has no effect on the

nearer to the terminal air tubes than the latter. Thus sudden and complete bronchoconstriction of vagal origin will have a greater effect on the intrapulmonary pressure and P.A.p. than tracheal occlusion in whatever phase of the respiratory cycle the obstruction to air entering and leaving the lungs takes place.

We attribute the fall of P.A.p. in fig. 5, *c*, *d*, to the fall of intrapulmonary pressure which accompanies the complete bronchoconstriction. These tracings should be compared with those obtained by tracheal obstruction (fig. 5, *e*). On the other hand, the rise of P.A.p. accompanying partial bronchoconstriction shown in fig. 7 cannot have been due to changes in the mean intrapulmonary pressure for the airway is only very slightly obstructed. The dynamic mean air volume has shifted towards the expiratory position, and this in itself may be sufficient to account for the rise of P.A.p. It would be expected that on occasion the rates of onset of complete bronchoconstriction giving rise to a new mean air volume and of the tendency for equalisation of the intrapulmonary pressure with the atmospheric pressure might be such that the effects on the P.A.p. nearly tend to balance one another, Slight differences in the temporal relations of these effects might therefore produce a diphasic P.A.p. response. We believe that fig. 5, *a*, illustrates such a mechanism. In this tracing the initial effect during one respiratory cycle of a reduction in T.A. towards the expiratory position would tend to produce a temporary rise of intrapulmonary pressure and of P.A.p. During the next cycle, at the end of which complete bronchoconstriction occurred at the mid-respiratory position, the intrapulmonary pressure would tend to equalise with the atmospheric pressure, thus causing a reduction of P.A.p. to its initial value. Thus the final P.A.p. value would be determined only by the position in which the lungs became fixed during complete bronchoconstriction. The fact that the P.A.p. changes lag considerably behind the bronchomotor effects is due to the large inertia of the mechanisms involved.

Changing Response of Pulmonary Arterial Pressure to Nerve Stimulation.—If we now assume that intrapulmonary pressure changes and the position in the respiratory phase at which the lung is immobilised are the only two mechanical factors responsible for passive P.A.p. alterations, then some of the P.A.p. responses to nerve stimulation we have obtained are apparently due to vasomotor effects. It has often been found that the relation between the P.A.p. and the accompanying bronchomotor response does not remain constant throughout an experiment. A description of one of these experiments will suffice to illustrate this point. Fig. 6 is a record of the effects of three strong stimulations each lasting 30 sec. of the L.C.V.S. nerve which successively gave a fall, no change, and a rise of P.A.p. (*a*, *c*, *e*). The fall of P.A.p. to the first stimulation appears to be of a permanent nature, a type of response we describe in a later paper as not unusual. It is accompanied by a

changing response of the pulmonary ganglia which relay the stimulated fibres to the periphery. A further interpretation is that the changing response of the bronchial muscle itself determines the final effect of nerve stimulation. We consider one of the two last mechanisms to be the most likely explanation.

In the experiments from which figs. 5 and 6 have been taken, the left lung had been removed prior to the acute experiment. The fact that L.C.V.S. stimulation causes a moderate bronchoconstrictor effect in one (fig. 5, *b*) and a weak bronchoconstrictor effect in the other experiment (fig. 6, *a*) indicates that the bronchomotor fibres are to some extent crossed. This confirms the results of previous workers [Dixon and Ransom, 1912; Braeucker, 1926]. That slight bronchodilatation can be produced by a second stimulation of the ipselateral C.V.S. nerve, the first stimulation having caused full bronchoconstriction (fig. 5, *c*), or by stimulation of the contralateral nerve after complete bronchoconstriction had been produced by stimulation of the ipselateral nerve, is of interest from one other point of view. Since only one lung is present, it rules out the possibility that the bronchodilatation is due to passive distension by bronchoconstriction of the opposite lung, this being a potential source of error in the interpretation of bronchomotor effects measured by lung plethysmography in the entire animal under positive pressure ventilation [see Dixon and Brodie, 1903; Weber, 1914].

Discussion and Conclusions.

Experimental evidence is produced supporting the view that in lungs under "negative" pressure ventilation the passive changes of P.A.p. accompanying bronchoconstriction of nervous origin are mainly due to changes in intrapulmonary pressure and in the degree of lung expansion. Intrapulmonary pressure changes are chiefly responsible for the P.A.p. alterations when the bronchoconstriction is sudden and complete, their direction being determined by the point in the respiratory cycle at which bronchoconstriction occurs. P.A.p. alterations due to partial or slowly produced complete bronchoconstriction are mainly due to changes in the degree of expansion of the lungs, which in turn determines the resistance of the pulmonary capillaries. In this interpretation of the effects of pulmonary nerve stimulation we have tentatively ascribed the diminution or cessation of tidal air excursions to bronchoconstriction. It may be, however, that the tidal air changes are due in part to a stiffening of the whole lung structure caused by an increase in the viscance and elastance of the lung tissues, for this was the effect of vagal stimulation on the lungs of the cat found by Bayliss and Robertson [1939]. If it is eventually found that canine lungs exhibit a somewhat similar vagal response, then our interpretations will require some revision in that the position in the respiratory

P.A.p. The rise of P.A.p. following the third L.C.V.S. stimulation is definite and occurs in the absence of bronchomotor effects, and we have no alternative but to interpret this response as being due to stimulation of vasomotor fibres. The fact that the third L.C.V.S. stimulation caused a rise of P.A.p. may have been due to the administration of adrenaline sensitising the peripheral nerve apparatus [see Burn, 1932]; this point requires further investigation. There is one other difference in the conditions between the first and third L.C.V.S. stimulations which requires mention. The systemic circulation blood-pump supplies blood to the bronchial vascular system, and if the communicating channels between the bronchial and pulmonary vascular systems are open, the transfer of blood from the former to the latter is responsible for a proportion of the pressure in the pulmonary artery [Berry and Daly, 1931]. The fall of P.A.p. due to the first L.C.V.S. stimulation may therefore have been due to constriction of the bronchial arteries. We do not think so, however, because the reduction in systemic arterial pressure when the pump was stopped should have caused a fall of P.A.p. if the communicating channels had been open. The P.A.p. rise due to the third L.C.V.S. stimulation could not have been due to *dilatation* of the bronchial arteries leading to an increase of the blood transferred to the pulmonary vascular bed for the reason that the systemic blood-pump had been stopped. Thus the P.A.p. rise in this case appears to have been due to a true pulmonary vasoconstriction.

The Nature of the Bronchomotor Response to C.V.S. Nerve Stimulation.—Stimulation of the caudal end of the C.V.S. nerves almost invariably produces bronchoconstriction. On a few occasions, however, a weak stimulus has caused slight bronchodilatation. Further, it has been found that when complete bronchoconstriction follows C.V.S. stimulation, a second stimulus of the same strength and duration temporarily releases the bronchoconstriction (fig. 5, *c*). Fig. 6 shows a somewhat similar phenomenon. In this experiment the left lung had been removed and strong stimulation of the contralateral L.C.V.S. caused only a weak bronchoconstriction (*a*), but when the stimulus was repeated (*c*) subsequent to a complete bronchoconstriction which had been produced by stimulation of the ipselateral R.C.V.S. nerve (*b*) it gave rise to a bronchodilatation. Several interpretations can be placed on these results. The C.V.S. nerves may contain bronchodilator fibres running in the cervical sympathetic portion as found by Dixon and Ransom [1912] and Saloz [1914] in the eserinised cat. Against this view are the results of Braeucker [1926] and of Daly, Elsden, Hebb, Ludány, and Petrovskaia [1942], who invariably obtained bronchoconstriction on stimulating the caudal ends of the separated cervical sympathetic nerves in the dog, an effect which was potentiated by eserine. Alternatively, such results as we have obtained, which are similar to those of Braeucker [1926] on the dog, may be due, as he has suggested, to the

of pulmonary vasomotor responses to nerve stimulation when broncho-motor changes also occur. It is true, however, that when nerve stimulation causes a P.A.p. change, the direction of which is opposite to that which may be expected to be produced by a concomitant broncho-motor change, it is suggestive of a pulmonary vasomotor response.

Summary.

Experiments on isolated perfused lungs and on the perfused whole animal preparation under "negative" pressure respiration are described. Attempts have been made to evaluate the bronchomotor mechanisms responsible for the changes of pulmonary arterial pressure (P.A.p.) which occur as a result of stimulation of the caudal end of the cervical vagosympathetic nerves (C.V.S.).

When complete bronchoconstriction occurs, its rate of onset, which determines the intrapulmonary pressure change, and the final position in the respiratory cycle in which the lungs are immobilised, are the main factors in determining the degree and direction of P.A.p. change. When partial bronchoconstriction takes place the P.A.p. change is chiefly governed by the direction of change of the mean air volume of the lungs.

Evidence is presented in support of the view that the C.V.S. nerves contain true pulmonary vasomotor fibres, but their functional activity cannot be unequivocally demonstrated by electrical stimulation if concomitant bronchomotor effects occur.

Experiments are described showing that C.V.S. stimulation may produce broncho-constriction or -dilatation. The conditions governing the type of response have not been evaluated.

We wish to express our thanks to the Government Grants Committee of the Royal Society for defraying the cost of the investigation by a grant to one of us (I. de B. D.).

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cycle in which the lungs are immobilised by vagal stimulation rather than changes in intrapulmonary pressure will be the important mechanical factor in determining the P.A.p.

Some of the observed changes of P.A.p. due to pulmonary nerve stimulation cannot be accounted for by passive effects; others take place in the absence of any bronchomotor response. We attribute the P.A.p. responses in these cases to stimulation of vasomotor fibres to the lungs. It is suggested that the vasomotor response which alters the P.A.p. may be a direct one on some portion of the pulmonary vascular bed, or an indirect one on some part of the bronchial vascular system. Daly and Euler [1932] have already shown that the bronchial vascular system is supplied with functionally active vasoconstrictor fibres. The P.A.p. is in part determined by the amount of blood transferred from the systemic circulation to the lesser circulation by way of the bronchial vascular system. Theoretically, therefore, constriction of the bronchial arteries should lead to a fall, and dilatation to a rise, of P.A.p. All our observations save one may be attributed to vasomotor effects on the bronchial or on the pulmonary vascular system. This exceptional observation, of a P.A.p. rise following C.V.S. stimulation, caused no bronchomotor effects and was made at a time when no blood flowed through the bronchial arteries. This suggests that vasoconstrictor fibres to the pulmonary vascular bed proper run in the C.V.S. nerves, and supports the earlier work of Cavazzani [1891] on isolated lungs perfused through the pulmonary artery without bronchial artery perfusion. The results of Daly and Euler [1932], however, which demonstrated vasoconstrictor effects of nerve stimulation in the absence of bronchomotor responses in lungs perfused through both the pulmonary and bronchial arteries, could be explained on the basis of true pulmonary vasoconstriction or of dilatation of the bronchial vascular system.

We have considered the possibility that vagal stimulation may produce contraction of the interstitial musculature of the lungs [Baltisberger, 1921] and so compress the lungs as a whole, an effect which when accompanied by complete bronchoconstriction might well raise the intra-alveolar pressure and compress the alveolar capillaries. Contraction of the interstitial musculature would undoubtedly alter the "hindrance" of the lungs and thereby cause a change in the inspiratory and expiratory levels of the tidal tracing. Since P.A.p. responses to nerve stimulation have been observed without any effect on the tidal air, we do not think that in these cases such a mechanism plays any part.

Although we feel that the results of this investigation clarify to some extent our knowledge concerning bronchomotor effects on the pulmonary arterial pressure, we are of the opinion that the complexity of the mechanisms *does not allow of the unequivocal demonstration*

EVALUATION OF BRONCHOMOTOR AND PULMONARY VASOMOTOR ACTIVITY BY MEANS OF THE PERFUSED LIVING ANIMAL UNDER NEGATIVE PRESSURE VENTILATION.

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Introduction.

The blood-perfused entire animal under negative pressure ventilation is a preparation in which the conditions are best suited for the unequivocal demonstration of pulmonary vasomotor activity. Its development in the form to be described in this paper originated in an endeavour to eliminate those physiological events which simulate pulmonary vasomotor activity but which are necessarily initiated under ordinary conditions by stimulation of the mixed nerve trunks in which the true pulmonary vasomotor fibres run. These vasomotor fibres proceed to the lungs by two main pathways—the cervical vago-sympathetic nerves and the upper thoracic sympathetic outflow—and it is probable that fibres from both sources run in the thoracic vago-sympathetic nerve trunk. Some of the nerve fibres supplying the heart and bronchi take a similar course; thus electrical stimulation of either pathway causes concomitant cardiomotor, bronchomotor, and pulmonary vasomotor effects. The associated changes in cardiac output and in bronchial calibre may simulate lung vasomotor effects by alterations in the blood-flow through the lungs and in the resistance of the pulmonary vascular bed [Bradford and Dean, 1889; Schafer and Lim, 1919; Schafer, 1921; Wiggers, 1921; Tigerstedt, 1923; Daly and Euler, 1932; Daly, 1933, 1936]. Their elimination is therefore an essential condition for the decisive demonstration of lung vasomotor activity.

Cardiac complications can be avoided by employing isolated perfused lungs. In such preparations stimulation of the stellate ganglion or of the *annulus Vieussenii* causes pulmonary vasoconstriction

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in the dog [Plumier, 1904], and weak constriction or dilatation in the cat [Tribe, 1914; Le Blanc and van Wijngaarden, 1924]. Excitation of the cervical vagus causes vasoconstriction in the rabbit [Cavazzani, 1891; Euler, 1932] and dilatation followed by constriction in the cat [Le Blanc and van Wijngaarden, 1924]. Stimulation of the cervical vagosympathetic trunk in the dog causes dilatation, and of the cervical sympathetic in the rabbit dilatation also [Cavazzani, 1891].

The main disadvantages of isolated perfused lung preparations are that the responses to vasomotor nerve stimulation even in fresh preparations are somewhat weak and can be obtained only for short periods. By additional perfusion of the bronchial vascular system to maintain viability of the bronchial muscles and intrinsic pulmonary nerve structures, Daly and Euler [1932] succeeded in obtaining a 30–70 per cent. increase of pulmonary arterial pressure by stimulation of the stellate ganglion. Excitation of the cervical vagosympathetic nerves produced only small changes of pulmonary arterial pressure. This result may have been due to loss of excitability of the stimulated portion of the nerve trunks since the blood-vessels to this region were not perfused. At that time it seemed evident that the only hope of investigating the function of these nerves under controlled conditions was to resort to perfusion of the entire animal and thus maintain the blood-supply to all the nerve pathways concerned. Preparations of this kind have been made by Dale [1928 *a*], Gibbs [1930], and Daly, Ludány, Todd, and Verney [1937], but detailed information of the general condition of the animal and of the state of the blood during perfusion was not published.

General Considerations.

In planning our method of perfusion of the entire animal our aim was to ensure that recorded changes in pulmonary arterial pressure were due solely to the effects of pulmonary vasomotor nerve excitation. For the demonstration of such effects we considered that the following criteria must be fulfilled:—

(1) *Constant Systemic Arterial Pressure.*—The pulmonary arterial pressure is in part determined by the quantity of blood transferred from the bronchial vascular system to the pulmonary circulation by way of the lung capillaries common to both systems. Thus a rise of pressure in the aorta and therefore in the bronchial arteries increases the quantity of blood so transferred and leads to an increase of pulmonary arterial pressure [Berry and Daly, 1931]. If the systemic pressure is constant, however, the transfer of blood is also constant provided the resistance of the bronchial vascular bed does not alter.

(2) *Constant Inflow of Blood to the Lungs.*—This condition, satisfied by perfusion of the lungs through the pulmonary artery, eliminates any change in pulmonary arterial pressure due to a simple change in inflow to the right ventricle. A separate motor for each blood-pump should be provided in order to prevent a change in load on one pump affecting the other.

(3) *Constant Pressure in the Right and Left Auricles.*—There is evidence that auricular pressure changes reflexly influence the resistance of the lesser circulation [Daly, Ludány, Todd, and Verney, 1937]. In our experiments this potential source of error was excluded by allowing the blood to flow freely from both auricles.

(4) *Negative Pressure Ventilation.*—This method of lung ventilation not only more nearly approaches the normal than positive pressure inflation, but also maintains the lungs in better condition.

(5) *The Maintenance of the Functional Integrity of the Pulmonary Nerves.*—Although systemic perfusion will give an adequate blood-supply to the nerves, the usual method of fixing the cannula in the pulmonary artery with a ligature will lead to damage of the pulmonary plexus and of the *glomus pulmonum* [Penitschka, 1931; Nonidez, 1935, 1936]. This is overcome by the insertion into the artery of a cannula fitted with an inflatable rubber sleeve to hold it in position [Morawitz and Zahn, 1912].

(6) *Œsophageal Movements as a Potential Source of Pulmonary Arterial Changes.*—Contractions of the œsophagus are caused by stimulation of the cervical vagus and may also occur spontaneously. Since it has been alleged that these movements may displace air from the lungs [Rugenberg, 1863; Gerlach, 1876; Beer, 1892; Grossmann, 1907], it is conceivable they might also change the pulmonary arterial pressure. It is therefore advisable to record œsophageal activity. In our experiments this was done by means of a rubber balloon inserted in the œsophagus as far as the level of the tracheal bifurcation.

(7) *The Separation of the Bronchomotor from Vasomotor Effects on the Pulmonary Pressure.*—Ever since de Jager [1885] showed that the pulmonary arterial pressure and blood-flow are in part dependent upon the intrapulmonary air pressure, it has been recognised that when stimulation of the pulmonary nerves causes changes both in bronchial calibre and in pulmonary arterial pressure, the latter effect is not necessarily due to excitation of pulmonary vasomotor nerve-fibres. It has been shown, however, in the dog [Daly and Euler, 1932]

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other method was to give initial intravenous injections of chloralose or chloralosane (0·05–0·1 g./kg. body-weight, sometimes mixed with urethane) and follow this with cyclopropane. In both methods the deep anæsthesia necessary for extensive surgical manipulation was not obtained until some cyclopropane had been administered; while for the rest of the experiment, as the effects of the initial anæsthetic became progressively less, the degree of anæsthesia depended entirely upon the amount of cyclopropane given. The method by which this could be adjusted is discussed in detail later. We are greatly indebted to Dr. J. Gillies for advice on cyclopropane anæsthesia.

Method.

Perfusion Apparatus and Operative Procedures.—The description which follows of the procedure for making the perfused preparation

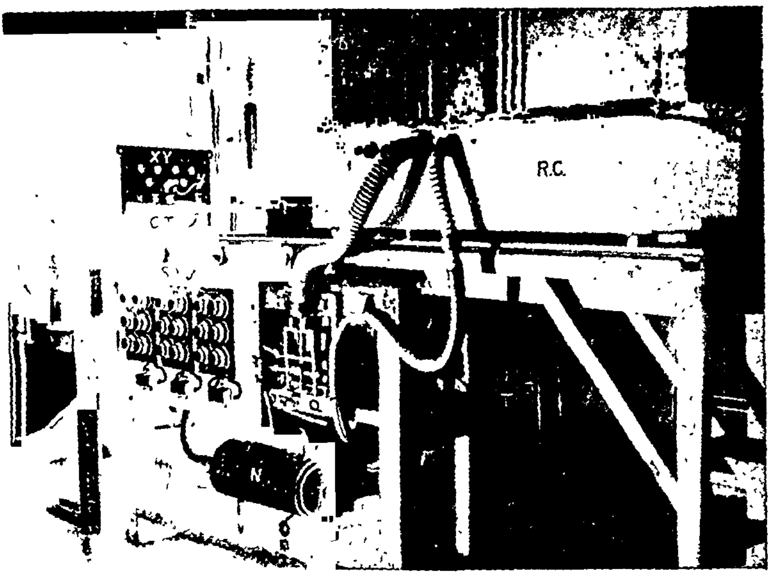

Fig. 1.—Apparatus showing operating tray (C.T.) and position of respiratory chamber (R.C.) during preliminary procedures. The lettering in this figure and in fig. 2 correspond. The switchboard controlling heating, lighting, and motors is shown at S.W.

is of the one finally adopted. The earlier experiments differed from it not only in the order of the operations but in the form of apparatus used, and when important these differences will be referred to in describing specific experiments.

One, two, or three dogs are bled to death from the femoral artery under local anæsthesia, and the blood ($1\frac{1}{2}$–$2\frac{1}{2}$ litres), after being rendered incoagulable, is kept at 37° C. For the test animal a dog of 3–10 kg. is selected, an animal of about 5 kg. being preferable with the apparatus

that pulmonary vasomotor effects may occur without any change in bronchial resistance. In the guinea-pig, the bronchial response to nerve stimulation may be so strong that it masks any vasomotor effect that may be present [Petrovskaia, 1939]. Under certain conditions, even in this animal, separation of bronchial and vasomotor effects is possible [Dale and Narayana, 1935; Hebb, 1940]. Success in this respect is generally obtained after all bronchomotor responses have failed.

The effects of bronchial calibre changes on the pulmonary arterial pressure in lungs under negative pressure ventilation have been discussed in the previous paper, and we propose to confine the present to a description of vasomotor responses which were uncomplicated by bronchomotor effects, or of which the bronchomotor accompaniments were minimal. It is true that the vasomotor responses obtained in many of our experiments were complicated by concomitant bronchial changes, but in some it was possible, by grading the strength of nerve stimulation, to obtain vasomotor responses in the absence of any tidal air change. Indeed in a few experiments the strongest stimulation failed to affect the bronchi while it still produced a definite vasomotor response.

Three other conditions we deemed essential for a successful perfusion: the first, that the animal should remain alive during perfusion and should not suffer from anoxia during the change over from the normal blood circulation to the perfused circulation; the second, that the state of the perfused blood should approach normal; and the third, that the depth of anæsthesia should be under control throughout the experiment. In the first of these aims we were successful, as will be shown; in the second, however, the necessity for using an anticoagulant imposed conditions the true significance of which in respect of our own results we are unable to assess.

With regard to the control of anæsthesia, our need was to find a method by which we could ensure deep anæsthesia during the preliminary surgical procedures and yet be able to maintain a lighter degree of anæsthesia during the perfusion period when the nerve responses were being tested. With intravenous methods the depth of anæsthesia cannot be readily adjusted, while in our experience chloroform or ether administered over long periods produces effects on the lungs which are incompatible with good experimental conditions. After trials of various methods we found two which gave satisfactory results: in one we used induction with ether most of which was subsequently blown off by the animal, and then followed this with cyclopropane admission into a closed circuit respiratory system. Our

electric motor, *m*, is shown by the arrows. A closed-circuit respiratory system is thus formed, in which the air circulator effects adequate absorption of carbon dioxide by the soda-lime. A continuous inflow of oxygen from a 50-litre bag, F, adjusted by the needle valve, *f*, replaces the oxygen consumed by the animal. In fig. 2 the tube connecting the trachea with tap T″ is shown, for the sake of clearness, passing through the side of the respiratory chamber. In practice it passes through the end plate XY at *y* (fig. 1), and is as short as possible so that there will not be too large a dead space.

Artificial respiration having been established, the motor driving the "Ideal" respiration pump is switched off and the large bore tap T″ turned 90° in a clockwise direction to enable the animal to blow off most of the ether. When it is evident that the animal is becoming less deeply anæsthetised the tap is turned back, the "Ideal" pump is started up and surgical anæsthesia obtained by the admission of cyclopropane from the cylinder (Cy). The amount of cyclopropane admitted is measured by a simple water-displacement device. In the event of an excess of cyclopropane being added, the gas mixture in the circuit can be diluted with oxygen after the withdrawal of gas by means of the piston in the brass cylinder, G, of 1 litre capacity. This piston, actuated by turning the handle, *g*, also serves to expel the explosive gas mixture from the cylinder to the outer atmosphere when the stopcock *t*′ has been turned 90° in a clockwise direction. Thus the whole operation of dilution is accomplished in less than a minute and can be repeated whenever necessary. The gas expelled from the cylinder is led outside the window of the laboratory to safeguard against explosions.

When the desired depth of anæsthesia has been attained the thorax is opened by splitting the sternum, the anticoagulant being injected into the femoral vein and cannulæ inserted into the cephalic ends of both femoral arteries and into the right and left auricles. The rubber tubes connecting these cannulæ to the perfusion pump are occluded. At this stage the cervical vagosympathetic nerves and the stellate ganglia are exposed for the later application of the stimulating electrodes. The two blood reservoirs (VR = pulmonary pump reservoir; V′R′ = systemic pump reservoir), each of length 25 cm., diameter 6 cm., and capacity 700 c.c., and the two Dale and Schuster [1928] pumps, P, P′, are then filled with blood. A Bayliss and Müller [1928] roller pump (R.P.) delivering approximately 600 c.c. per minute is set in motion for the purpose of circulating blood from the reservoir VR through a filter (*h*), and thence through a Hooker [1915] and Drinker [1922] type of oxygenator (Ox) to the reservoir V′R′, whence any excess spills over into the reservoir VR. The oxygenator is fed with oxygen gas, sometimes with the admixture of carbon dioxide. The concentrations of carbon dioxide used were 7 or 10 per cent. as required. The filter *h*

we have used. The animal, anæsthetised with ether or chloralosane, is placed on an electrically heated copper tray (fig. 1, C.T.), a tracheal cannula is inserted and the lungs are rhythmically inflated with a Starling "Ideal" respiratory pump [Starling, 1926]. This pump, A (fig. 2), delivers oxygen from the bag, B, to the lungs through the

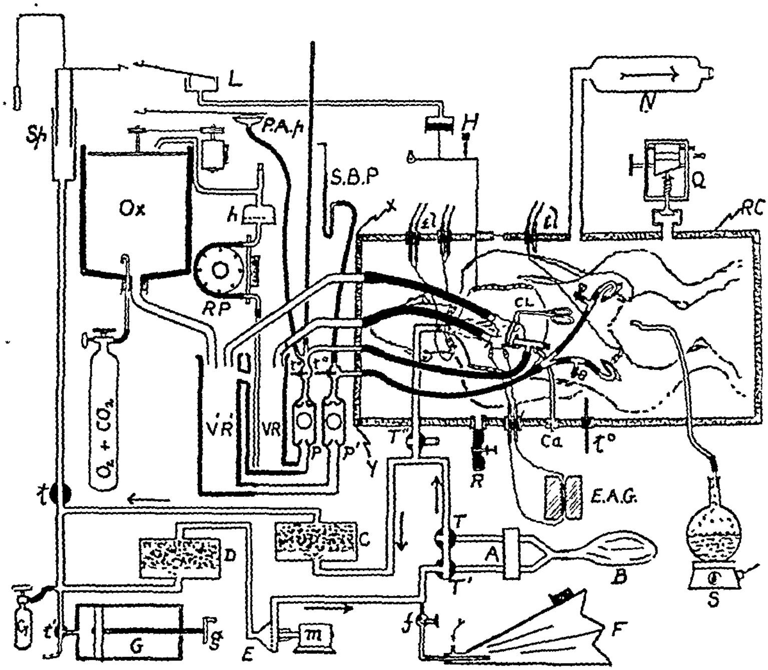

FIG. 2.—Diagram of perfused whole animal with negative pressure ventilation.

A = Starling "Ideal" respiration pump; B = rubber bag; C and D = soda-lime chambers; Ca = compressed air supply; CL = heart clamp; Cy = cyclopropane cylinder; E = air circulator driven by motor (m); E.A.G. = string galvanometer for electroauriculogram; el = stimulating electrode connections; F, f = 50-litre gas bag and needle valve; G = cylinder of adjustable capacity by rotation of g; h = blood filter; H = piston recorder; L = water-volume recorder; N = vacuum cleaner; Ox = oxygenator; P.A.p. = pulmonary arterial pressure manometer; P, P′ = Dale and Schuster blood pumps; Q = poppet-valve; R = escape valve; RC = respiratory chamber; R.P. = roller pump; S = steam kettle; S.B.P. = Hg. manometer; Sp = spirometer: T, T′, T″, t, t' = taps; $t°$ = thermometers; VR = pulmonary pump reservoir; V′R′ = systemic pump reservoir; XY = end-plate of respiratory chamber.

wide bore tap, T, which is turned 90° in a clockwise direction from the position shown in the figure. During the suction stroke of pump A, when the pump cylinder is being filled with oxygen from bag B, the lungs collapse by virtue of their own elasticity and the expired gases pass through the soda-lime containers C and D back to the pump A: the tap T′ at this stage of the experiment is 90° anticlockwise from the position shown, and the tap t leading to the spirometer (Sp) is closed. The direction of gas-flow, aided by an air circulator, E, driven by an

consists of 100–150 μ mesh muslin (when dry) with an area of 30 sq. cm. Nearly full saturation with oxygen of the blood in the reservoir V′R′ is achieved, and with the CO_2 mixtures the CO_2 content of the blood reaches 50 vols. per cent. The pump P′ is then set going and the blood from V′R′ fed to the systemic circulation of the animal through the femoral arteries, the artery forceps on the tubing of the right auricular cannula having been removed. The blood returns from the right auricle to the reservoir VR to be once more oxygenated. During this stage the heart is still beating strongly, and the blood-pressure is maintained between 100 and 160 mm. Hg by adjusting the output of the pump P′.

Perfusion of the systemic circulation having been established, an incision is made in the right ventricle, and a cannula (fig. 3), connected to the output of pump P, is passed through the opening into the pulmonary artery. This cannula is similar in design to a Morawitz and Zahn [1912] coronary sinus cannula, its peripheral end being covered by a rubber sleeve, inflation of which with compressed air supplied through the tube Ca firmly locates the opening of the cannula in the proximal end of the pulmonary artery. A specially designed heart clamp (CL, figs. 2 and 3) is then applied round the ventricles below the auriculo-ventricular junction; this compresses the ventricle musculature firmly round the P.A. cannula, putting both ventricles out of action and preventing any auricular blood reaching their cavities. Care is taken to ensure that the clamp does not interfere with the blood reaching the auricles. During the short time necessary for insertion of the P.A. cannula the output of the systemic pump P′ is adjusted so that the systemic blood-pressure is maintained at a high level. The insertion of the cannula and adjustment of the clamp take 30–120 seconds (fig. 4).

The pump P for perfusion of the lungs is started up after removal of the clamp on the tubing of the left auricular cannula, and the oxygenated blood from the left auricle collected in the reservoir V′R′ to mix with the oxygenated blood from the oxygenator (Ox). The blood now entering the lungs generally causes some pulmonary vasoconstriction, which is gradually relieved as the blood recirculates (see fig. 7). In order to prevent damage to the lungs at this time the initial output of pump P is kept low, but as the vasoconstriction wears off it is increased. The systemic arterial pressure and pulmonary arterial pressure (P.A.p.) levels are maintained at 120–180 mm. Hg. and 25–40 cm. saline respectively by adjustment of the pumps. Generally the pulmonary flow is somewhat smaller than the systemic, since there is some residual pulmonary vasoconstriction.

As soon as both artificial blood circulations are satisfactorily established, the main portion of the respiratory chamber (R.C.) is run up on rails to be fixed by four butterfly nuts to the end-plate XY (fig. 1).

Thus the animal lies totally enclosed in the chamber, except that the top is still open. Electrodes are then placed on the nerves to be stimulated. A fine hook is attached to the chest wall from which a thread passes through a hole $\frac{1}{32}$ inch in diameter to a piston recorder (H, fig. 2) connected by air transmission to a water volume recorder (L) for the purpose of registering respiratory efforts.

The auricles keep beating, since their blood-supply is intact (fig. 5). The wires from the stimulating electrodes pass to fittings located in

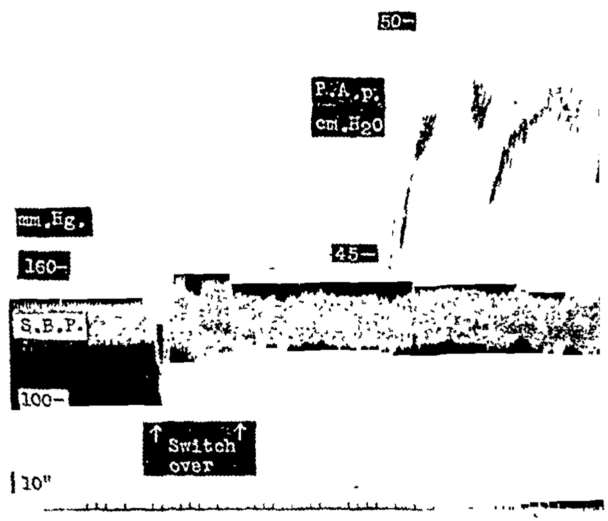

FIG. 4.—Expt. 51. Dog, ♀, 4·7 kg. Chloralose-cyclopropane. At the beginning of the tracing the systemic arterial pressure is maintained by the animal's heart and by the output of the systemic pump. During the insertion of the pulmonary arterial cannula and clamping of the heart (between arrows) the systemic output of the pump is adjusted to maintain the blood-pressure.

rubber bungs which are inserted in the wall of the chamber. The wires carrying the stimulating current are connected to these by means of banana plugs (*el*). Respiration and auricular frequency have not been recorded in all the experiments. The auricular frequency is registered by means of a string galvanometer (E.A.G.) which records the electroauriculogram.

The animal is now switched over from positive pressure lung inflation to negative pressure respiration. To do this the top of the respiratory chamber is covered with a sheet of plate-glass, and the joint is made air-tight with a high melting-point vaseline. The Vactric vacuum cleaner (N), for continuously exhausting air from the chamber, and the motor driving the variable cam poppet-valve (Q) [Daly and Thorpe, 1930] for intermittently letting air into the chamber, are

FIG. 3.—Two sizes of pulmonary arterial cannulæ and of heart clamps. × $\frac{1}{3}$.

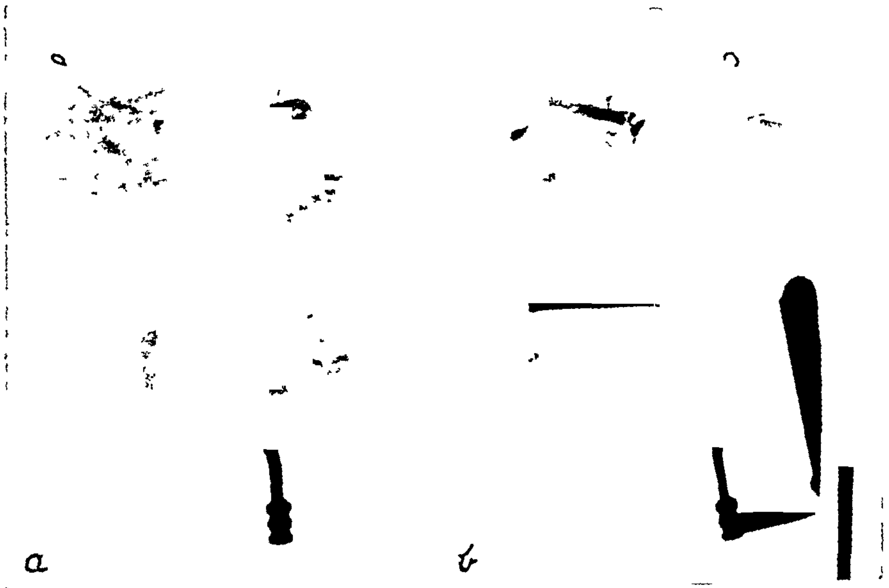

FIG. 5.—Radiograph. Cannula and heart clamp in position in heart, the coronary vessels of which were injected with 40 per cent $BaSO_4 + 12\frac{1}{2}$ per cent gelatin. A circlet of wire stitched to the sino-auricular node is shown in *a* (top left) and in *b* (partly covering shadow of cannula tip). The coronary arterial vessel to the *s–a* node is well shown in *a*.

a = antero-posterior view. *b* = lateral view.

DALY, ELSDEN, HEBB, LUDÁNY, and PETROVSKAIA, "Bronchomotor and Pulmonary Vasomotor Activity."

copper wire wound on an iron core of $\frac{5}{8}$ inch diameter, and a secondary of 7500 turns of 38 S.W.G.; the primary was fed from a 4-volt accumulator. The frequency of stimulation has ranged between 60 and 80 per second. In others, the same primary and secondary coils have been used with a frequency of 38·5/sec. intermittently applied,

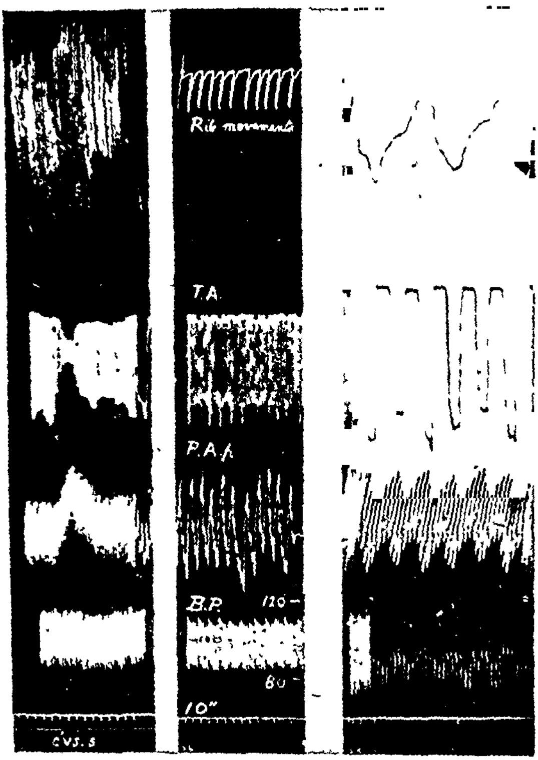

FIG. 6.—Expts. 19, 24 and 25. Dogs. P.W.A., N.P.V. Respiratory movements of the ribs. T.A.=tidal air (due to negative pressure ventilation), P.A.p.=pulmonary arterial pressure, and B.P.=systemic blood-pressure of perfused whole animal. Phrenic nerves intact in all experiments.

In Expt. 19 stimulation of the caudal ends of both cervical vagosympathetic nerves causes a rise of pulmonary arterial pressure and bronchoconstriction.

P.W.A.=perfusion of whole animal; N.P.V.=negative pressure ventilation.

In all figures, a downward excursion of the T.A. tracing indicates lung expansion.

the ratio of period of stimulation to period of intermission being 5 to 1, 4 to 1, 2 to 1, or 1 to 1. The stimulating electrodes consist of platinum-wire hooks inserted into a piece of rubber tubing 2 cm. in length slit longitudinally, which serves to guard the electrodes from the surrounding tissues.

In the dog the separation of the cervical sympathetic from the cervical vagus requires careful dissection if the functional integrity of the nerves is to be preserved. Provided the operation is not carried out hurriedly success is usually attained, although no doubt the

switched on. At the same time the motor driving the "Ideal" respiration pump (A) is switched off, and the large bore taps T, T′ and *t* are rotated into the positions shown in fig. 2. The switches and taps are so arranged that these operations can be carried out in a few seconds. An adjustable screw-clip on a wide bore rubber tubing (R) controls the amount of air leaking into the chamber, and thus regulates the depth of respiration. Steam is passed into the chamber, the amount being controlled by an electric heater (S). The draught of air through the chamber causes rapid condensation of the steam, and thus serves to keep the lung surfaces moist without danger of overheating them. The tidal air is recorded on the kymograph paper by the spirometer (Sp).

The tray on which the animal lies is electrically heated with carbon lamps. These lamps also keep the main chamber warm after it has been located on the end piece XY. A separate group of heaters is used for warming the main chamber during the preliminary stages of the operation. A bank of 18 iron-clad switches (SW, fig. 1) controls the lighting, heating, and motor gear, thus eliminating the danger which sparking at switch contacts might cause in the event of an escape of cyclopropane outside the closed air-circuit system. The soda-lime is in two containers (C, D, fig. 2), which present approximately equal resistance to the air current produced by the air circulator (E). The spirometer (Sp) is connected to the air output of one container and the input of the other, the air circulator being situated between the two remaining airways. This system is adopted to ensure a well-balanced spirometer.

The blood used for perfusion is rendered incoagulable either by whipping or by the addition of heparin (Jorpes, B.D.H., 10–17 mg./100 c.c. blood); and the use of such blood has probably been the main reason for the production of the abnormal background of some of the preparations. The improved arrangements of perfusion and the increasing ease with which the preparations have been made as the investigation progressed militate against a satisfactory comparison being made of the relative merits of the two perfusates. We have gained the impression, however, that the use of heparin is preferable to defibrinating the blood. In a few preparations, both "vasotonins" [Janeway, Richardson and Park, 1918; Eichholtz and Verney, 1924] and "bronchotonins" [Daly, 1938] were conspicuous by their absence.

The necessity for careful blood filtration in perfusion experiments has been shown by Anrep and Hausler [1928], Daly and Thorpe [1933], Evans, Grande and Hsu [1934], and Bogue and Gregory [1939]. We therefore filter the blood several times after collection from the donors and insert a filter in the perfusion apparatus.

Preparation of Nerves for Stimulation.—In some experiments the nerves have been stimulated by means of an induction coil (C. F. Palmer & Co.) which consists of a primary of 300 turns of 20 S.W.G.

preparation for perfusion, which was not, however, carried out in this experiment. Anæsthesia consisted of ether induction alone, which was deeper than that usually practised, and consequently it was not necessary to add any cyclopropane or more ether once the animal had been connected to the closed circuit respiratory system. Table I. shows the course of the experiment. The O_2 consumption was measured by the slope of the T.A. tracing—the O_2 to the closed circuit system being cut off temporarily during each measurement (see fig. 20, *d*). A second experiment gave similar results. The air circulator and removal of carbon dioxide by the soda-lime were therefore efficient.

General Course of Perfusion Experiments.—Forty experiments were performed in which stimulation of the pulmonary nerves or drug injections caused changes in the lungs. In the first 23 experiments the lungs were not shunted by an oxygenator, and there is no doubt that in these some degree of anoxia and CO_2 retention was present, especially when bronchoconstriction resulted from the nerve stimulations. Further, the expansion of the lungs under negative pressure ventilation was frequently inadequate, due, we suspect, to the use of defibrinated blood as the perfusate. In the remaining 17 experiments the oxygenator was in circuit, and in 3 of these heparin was used as an anticoagulant. The presence of the oxygenator gave us a separate control of the blood O_2 and CO_2 content irrespective of the state of the lungs. Moreover, when this series was started major difficulties in technique had already been overcome, and the success attending the experiments was partly due to this factor. In 6 of these gas analyses of the blood were made, and showed good oxygenation of the blood perfusing the systemic circulation in 3 experiments and a CO_2 content above 45 vols. per cent. in 4 experiments. Some preparations therefore were working under anoxic and/or acapnic conditions. Although we achieved success in keeping the gaseous content of the blood within normal limits in our later experiments (see fig. 9), we should mention that in the majority of the earlier experiments this was probably not so.

Respiratory centre activity persisted up to 3 hours of perfusion in some experiments, even when no oxygenator was present in the circuit. In others it failed as soon as the perfusion was started, probably in part due to the low CO_2 content of the reservoir blood which had not circulated through the animal. Evidence that this was so was provided by some later experiments in which the difficulty was apparently overcome by passing the reservoir blood through the "oxygenator" fed with O_2 and 5 per cent. or 10 per cent. CO_2 just before perfusion was started. It often happened too that irregular respiratory rhythm or apnœa appeared temporarily (fig. 7). In yet other experiments respiratory activity would cease quite early on in the perfusion and fail to reappear. The elucidation of these changes will require continuous blood gas sampling.

function of the nerves is impaired. In the earlier experiments the fibres, once separated, were stimulated with a weak current and the separation confirmed by observation of the effects upon the eye and heart. Stimulation of the cephalic end of the C.S. nerves causes dilatation of the pupil, retraction of the nictitating membrane and widening of the palpebral fissure. Later in the investigation the tests were not always done, as the experience of past experiments enabled us to recognise the two separate nerve bundles by their characteristic appearance. In most of the dissections fibres passing between the two nerve bundles had to be cut.

Stimulation of the stellate ganglion is carried out by placing the electrodes directly on the ganglion itself, its connections with the spinal nerves and thoracic sympathetic chain being usually cut but sometimes left intact.

In many experiments one or both of the phrenic nerves have been cut in order to prevent alterations in the mean air volume of the lungs caused by diaphragmatic movements due to the animal's respiratory efforts (fig. 6). Even when both phrenic nerves are cut, vigorous respiratory efforts on occasion cause sufficient movement of the thoracic walls to displace some air into and from the lungs. These movements produce slight alterations in the tidal air tracing.

Results.

Closed Circuit Respiratory System.—This was tested separately in an animal under negative pressure ventilation and connected to the closed circuit respiratory system, the thorax being opened as if in

Table I.

D.H. 17. *Dog, ♀, 9·9 kg. Morphine sulphate 16 mg. Ether. Natural circulation.* Test of closed circuit respiratory system. Thorax opened; negative pressure ventilation.

Time.	H.F. per min.	B.P. mm. Hg.	Respiration per min.	O_2 cons. c.c./min.
11.20 a.m.	176	..	16	..
11.21 a.m.	Thorax opened. Positive pressure ventilation.			
12.40 p.m.	200	100	(11)	..
12.57 p.m.	Negative pressure ventilation.			
1.06 p.m.	168	107	(9)	69
1.44 p.m.	..	100	(16)	..
1.58 p.m.	..	95	(11)	..
3.07 p.m.	144	96	(16)	..
3.41 p.m.	..	90	(14)	75

H.F. = frequency of heart-beat. The figures in brackets indicate the frequency of respiratory efforts after the thorax had been opened and lung ventilation artificially maintained.

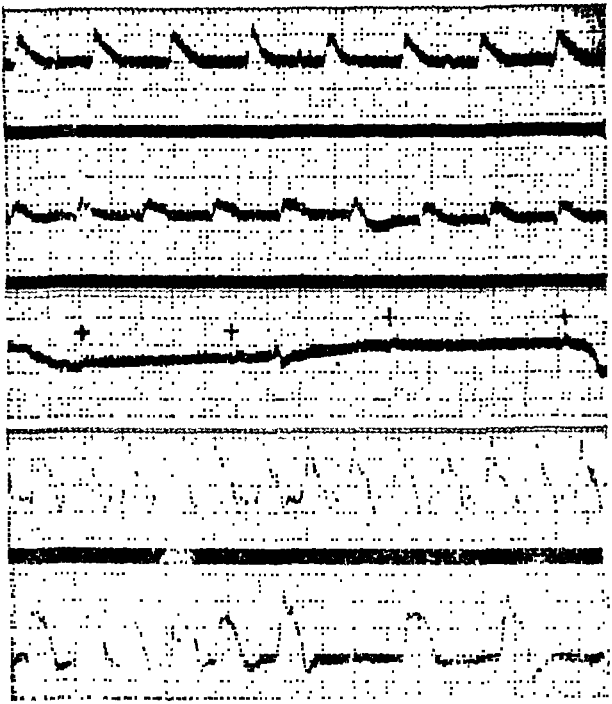

FIG. 8.—Expt. 45. Dog, ♂, 9·6 kg. P.W.A., N.P.V. Chloralosane. Oxygenator in circuit. Electroauriculogram (E.A.G.) taken, 62′, 114′ and 123′ after start of perfusion. The larger waves 123′ after perfusion are due to displacement of the lungs during ventilation. The smaller waves marked with a cross are due to auricular beats.

D.E.H. 46/2. Dog, ♀, 3·4 kg. P.W.A., N.P.V. Chloralosane-cyclopropane. Oxygenator in circuit. E.A.G. 34′ and 109′ after start of perfusion; the latter taken at commencement of stimulation of caudal ends of C.V.S.

Time $=\frac{1}{5}$ and $\frac{1}{25}$ sec.

TABLE II.—PERFUSION BLOOD-FLOWS IN DOGS UNDER NEGATIVE PRESSURE VENTILATION.

Expt.	Body-wt., kg.	B.P., mm. Hg.	P.A.p., cm. sal.	Systemic flow, c.c./min./kg.	Pulmonary flow, c.c./min./kg.	Blood.
35	6·9	135	22	47	31	D.B.
41	8·7	240	20	76	69	D.B.
42*a*	9·2	105	29	78	33	D.B.
42*b*	9·2	125	42	50	20	D.B.
43*a*	6·9	115	22	28	54	D.B.
43*b*	6·9	160	20	71	69	D.B.
45	9·6	150	27	40	43	D.B.
46	3·4	115	34	85	35	D.B.
48	6·5	110	20	80	102	D.B.
49	6·0	140	33	63	63	H.B.
50	8·7	70	15	87	46	H.B.

D.B. = defibrinated blood; H.B. = heparinised blood.

The auricles generally continued to beat for 1–3 hours after the start of perfusion, and responded by slowing or stopping to stimulation of

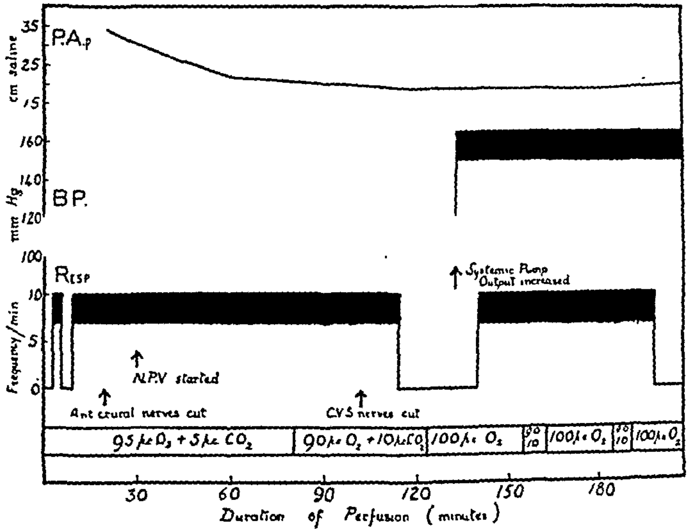

Fig. 7.—Expt. 43. Dog, ♀, 6·8 kg. P.W.A., N.P.V. Oxygenator in circuit. Ether-chloralosane-cyclopropane. Phrenic nerves cut. Stellate ganglia and thoracic sympathetic chains intact.

From above downwards: mean pulmonary arterial pressure; limits of systemic arterial pressure and of respiratory frequency and the gaseous mixture supplied to the oxygenator.

N.P.V. = Negative pressure ventilation.

the peripheral end of the C.V.S. (cervical vagosympathetic) or C.V. (cervical vagus) nerves (fig. 8).

Measurements of the outflowing blood from the right and left auricles in 9 experiments are given in Table II. They are only approximate, the measurements being made simply by allowing the auricular blood to drain into a measuring cylinder for periods of 5 seconds or 10 seconds and are probably subject to an error of ± 15 per cent. The flows given are on the low side, which is surprising in view of the fact that the recorded systemic blood-pressures are mostly within normal limits; the pulmonary pressures, however, tend to be on the high side, although at the time the flow through the lungs was deliberately kept low in order to prevent damage to the blood-vessels and the onset of lung œdema.

The minute volume for the blood-flow of the unanæsthetised dog is given by Marshall [1926] as 130 c.c./min./kg. (mean of 5 animals). The presence of vasotonins in the preserved blood may be responsible for the low flows in our experiments.

Dr. O. A. Trowell kindly carried out a histological examination of the liver, spleen, kidney, intestine, suprarenals, skeletal muscle, and

the anoxic and well-oxygenated preparations, as well as in the hyper- and hypo-capnic preparations; it ranged from 6·76 to 7·40. Anæsthetics might have some responsibility for this, as it has been

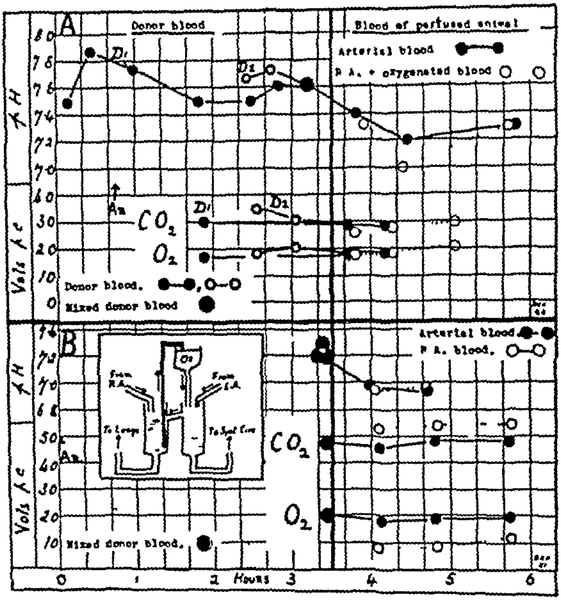

FIG. 9, A.—Expt. 46. Dog, ♀, 3·4 kg. P.W.A., N.P.V. Chloralosane-cyclopropane. Both phrenic and both C.V.S. nerves cut; thoracic sympathetic nerves intact. Upper left half of graph shows change of pH, O_2 and CO_2 content of incubated defibrinated blood of two donor dogs (D1, D2) during the time the test animal was being prepared for perfusion. Upper right half shows blood changes during 2¼ hours' perfusion with donors' and test animal's blood. Perfusion started at 3½ hours. Respiratory centre inactive during perfusion. The R.A. (right auricle) +oxygenated blood was taken for analysis from the pulmonary pump reservoir (left-hand reservoir, inset diagram). The arterial blood sample was taken from the systemic pump reservoir (right-hand reservoir). The relative positions of the reservoirs are drawn reversed in this figure as compared with fig. 2. *An.* indicates point in time when anæsthetic was given to test animal.

L.A.=left auricle. Systemic blood-flow 298 c.c./min.; pulmonary flow 120 c.c./min. Oxygenator pump blood-flow=500–600 c.c./min. Oxygenator fed with 99 per cent. O_2.

FIG. 9, B.—Expt. 51. Dog, ♀, 4·6 kg. P.W.A., N.P.V. Chloralose-cyclopropane. *Left thoracic sympathethic chain and stellate ganglion removed* 71 *days previously. Right lung and right thoracic sympathetic chain with stellate ganglion removed* 48 *days previously.* R. phrenic nerve and both C.V.S. nerves cut. L. phrenic nerve intact. Similar type of experiment to Expt. 46 except that oxygenator was fed with 90 per cent. O_2+10 per cent. CO_2. Perfusion started at 3½ hours. The blood from R.A. was taken from R.A. *tube* entering left-hand reservoir (inset). The donors' blood was heparinised.

shown that cyclopropane may diminish the blood pH [Fay, Andersch and Kenyon, 1939] though ether apparently does not do so [Wunsche, 1937]. Yoshimura [1935] reported that heparin (1·0 per cent.) has no influence on the pH of shed blood, and it appears unlikely therefore that this anticoagulant caused the low pH in our experiments.

lungs in Experiments 48, 49, and 50. Marked congestion of the liver and spleen (Expt. 50) and of the intestinal villi (Expt. 48), and some congestion of the kidney (Expts. 49 and 50), was present. The lungs showed some extravasations of blood in the adventitia of the larger blood-vessels (Expt. 48) and were relatively bloodless. The number of experiments is too small, however, to warrant an attempt to correlate these findings with the blood-flows and duration of perfusion.

The State of the Blood during Perfusion.—In 5 experiments we followed the change in pH of the donor's blood during incubation whilst the test animal was being prepared for perfusion. The determinations were made by a Beckman electrometric apparatus using 3 c.c. of blood. The blood taken from the femoral artery under local anæsthesia was defibrinated in 3 of these experiments and heparinised in 2 (14·0 and 17·0 mg. heparin/100 c.c. blood). The pH taken within a few minutes of bleeding ranged between 7·40 and 7·75. In 1 experiment the determination was made on blood taken straight from the artery. During the subsequent 3 hours of incubation the pH rose, fell, or remained constant (fig. 9, A). The pH of the mixed donor blood used to fill the perfusion apparatus varied from 7·50 to 7·70, but in a further experiment in which the blood was passed through the oxygenator fed with 90 per cent. O_2 + 10 per cent. CO_2 just before perfusion was started, the pH was reduced to 7·20 (fig. 9, B). Berg, Mayne and Petersen [1940] reported that the pH of dog's plasma varies from 7·32 to 7·68 and is probably determined in part by the barometric pressure.

Blood gas and pH determinations during perfusion were made in 6 experiments and in 3 of these both the O_2 and CO_2 content of the arterial and venous bloods were within normal limits. The blood gases were determined by van Slyke's method (fig. 9). The oxygen and carbon dioxide contents of the arterial and mixed venous blood in normal intact dogs have been measured by Stewart [1924]. His results from not less than 18 animals in each case were as follows: vol. O_2 per 100 c.c. of arterial blood ranged from 11·42 to 22·02 (mean, 19·06); per 100 c.c. of mixed venous blood, 8·57 to 18·45 (mean, 15·44). The values for vol. CO_2 were 34·04 to 48·93 (mean, 43·1) and 42·04 to 56·12 (mean, 47·32) per 100 c.c. of arterial and of mixed venous blood respectively.

In one of our experiments the systemic pump reservoir blood which is delivered to the systemic circulation had a lower pH than that of the pulmonary pump reservoir blood which is delivered to the lungs and oxygenator, in spite of the fact that the former blood had a lower CO_2 content than the latter. We are uncertain as to the meaning of this discrepancy, for the conditions determining the nature of the blood in the two reservoirs are somewhat complex owing to the manner in which blood is transferred from one to the other. The pH of the arterial blood was generally lower than the expected value both in

an accompanying bronchodilatation it occurred synchronously with the rise in P.A.p., and was due therefore to dilatation of the terminal passages of the bronchial tree, which are alone supplied by the pulmonary circulation. That the rise in systemic pressure was due to recirculation of the adrenaline is suggested by the long latent period which was

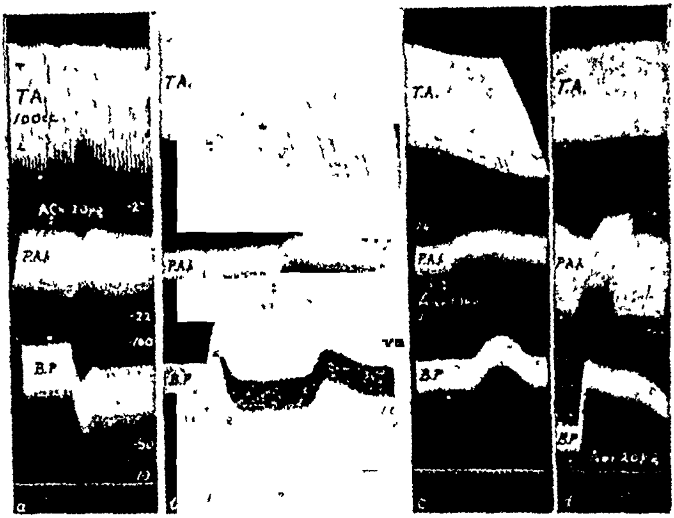

FIG. 11, *a*.—Expt. 25. Dog, ♀, 6·7 kg. P.W.A., N.P.V. No oxygenator. Ether-cyclopropane, eserine 4·0 mg. Phrenic nerves intact; both C.V.S. nerves cut. Thoracic sympathetic nerves intact. Effect of ACh. 20 μg. injected into pulmonary arterial tubing.

b. Expt. 35. Dog, ♂, 6·8 kg. P.W.A., N.P.V. Oxygenator in circuit. Chloralose-cyclopropane, eserine 2·0 mg. Both phrenic and both C.V.S. nerves cut; thoracic sympathetic nerves intact. Systemic blood-flow 330 c.c./min.; pulmonary flow 210 c.c./min.

1 = adrenaline (10 μg.) injected into systemic arterial tubing.
2 = adrenaline (10 μg.) injected into pulmonary artery tubing.

c. Expt. 35. Effect of adrenaline (10·0 μg.) injected into pulmonary artery tubing 6 minutes after *b*, 2.

d. Expt. 45. Dog, ♂, 9·6 kg. P.W.A., N.P.V. Oxygenator in circuit. Chloralosane. Both phrenic and both C.V.S. nerves cut. Thoracic sympathetic nerves intact. Systemic blood-flow = 380 c.c./min.; pulmonary flow = 410 c.c./min. Effect of adrenaline (20·0 μg.) added to systemic pump reservoir.

not significantly altered in value as a result of a second dose of adrenaline injected into the pulmonary arterial tubing (fig. 11, *c*). This interpretation is supported by the data presented in fig. 11, *a*. In this experiment 20 μg. of acetylcholine injected into the pulmonary artery tube caused an immediate bronchoconstriction but no significant change in the B.P. or P.A.p. After a latent period of 37–38 seconds the B.P. fell synchronously with a slight diphasic change of P.A.p. These later effects were evidently due to the ACh. reaching the peripheral systemic circulation and the lungs by way of the bronchial circulation respectively. That the recirculating ACh. had reached the

The Action of Adrenaline.—In 6 experiments in which Pernocton was used as an anæsthetic, adrenaline was infused at a steady rate into the systemic circulation at rates of 5, 9, 11, 12, 15, and 18 μg./min./kg. body-weight. The larger quantities raised the systemic arterial pressure and caused gross irregularities lasting during the infusion (fig. 10); the smaller quantities, with the exception of 5 μg./min./kg., produced only transient irregularities in pressure level. An insufficient number of experiments has been performed with

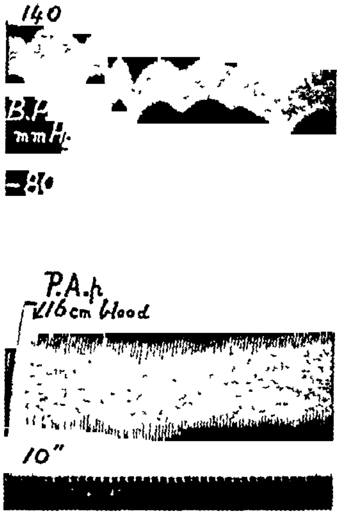

Fig. 10.—Expt. 6. Dog, 7·0 kg. P.W.A. Positive pressure ventilation. No oxygenator. Pernocton 0·5 c.c./kg. body-weight. Phrenic nerves, stellate ganglia and sympathetic chains intact. C.V.S. nerves cut. Irregular systemic arterial pressure due to adrenaline infusion 15 μg./min./kg. body-weight. At signal—stimulation of caudal ends of both cervical vagosympathetic nerves.

adrenaline infusions to decide whether the preparations are improved by this practice. Freeman, Freedman and Miller [1941] have recently found that adrenaline infused at 3·4–16·4 μg./min./kg. body-weight causes a state of shock, an increase of venous pressure and a decrease in plasma volume. The advisability of practising adrenaline infusion clearly requires further examination, especially as Stutzman and Allen [1941] have reported that cyclopropane has an adrenolytic action in the later stages of anæsthesia.

Single injections of adrenaline (5–20 μg.) in preparations anæsthetised with chloralosane-cyclopropane gave interesting results depending upon the point in the circulatory system selected for injection. When injected into the pulmonary artery tubing the rise in P.A. pressure was almost immediate, and after a latent period which varied from 25 to 70 seconds in a number of experiments a rise in systemic arterial pressure occurred (fig. 11, *b*, *c*). When there was

an accompanying bronchodilatation it occurred synchronously with the rise in P.A.p., and was due therefore to dilatation of the terminal passages of the bronchial tree, which are alone supplied by the pulmonary circulation. That the rise in systemic pressure was due to recirculation of the adrenaline is suggested by the long latent period which was

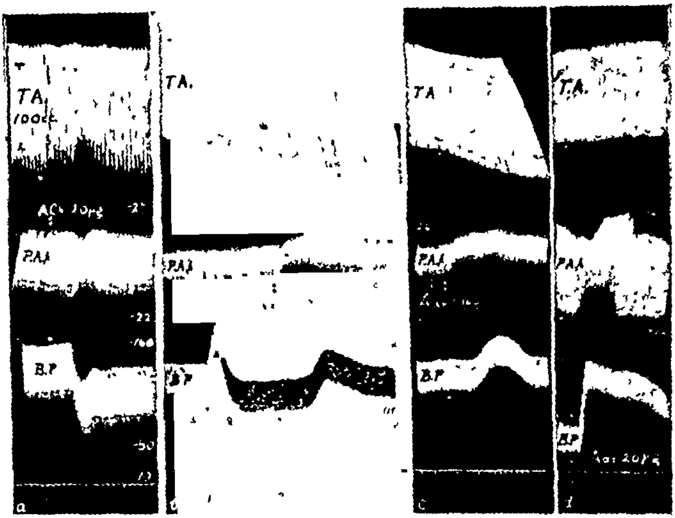

FIG. 11, *a*.—Expt. 25. Dog, ♀, 6·7 kg. P.W.A., N.P.V. No oxygenator. Ether-cyclopropane, eserine 4·0 mg. Phrenic nerves intact; both C.V.S. nerves cut. Thoracic sympathetic nerves intact. Effect of ACh. 20 μg. injected into pulmonary arterial tubing.

b. Expt. 35. Dog, ♂, 6·8 kg. P.W.A., N.P.V. Oxygenator in circuit. Chloralose-cyclopropane, eserine 2·0 mg. Both phrenic and both C.V.S. nerves cut; thoracic sympathetic nerves intact. Systemic blood-flow 330 c.c./min.; pulmonary flow 210 c.c./min.

1 = adrenaline (10 μg.) injected into systemic arterial tubing.
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not significantly altered in value as a result of a second dose of adrenaline injected into the pulmonary arterial tubing (fig. 11, *c*). This interpretation is supported by the data presented in fig. 11, *a*. In this experiment 20 μg. of acetylcholine injected into the pulmonary artery tube caused an immediate bronchoconstriction but no significant change in the B.P. or P.A.p. After a latent period of 37–38 seconds the B.P. fell synchronously with a slight diphasic change of P.A.p. These later effects were evidently due to the ACh. reaching the peripheral systemic circulation and the lungs by way of the bronchial circulation respectively. That the recirculating ACh. had reached the

lungs was shown by a further bronchoconstriction which occurred after the fall in systemic B.P. These observations extend those made by Alcock, Berry, Daly, and Narayana [1936], who, working on lu-ng œsophagus preparations, compared the effects on the bronchi of adrenaline injected into the bronchial and pulmonary circulations.

Single doses of adrenaline (10–20 μg.) injected into the systemic arterial tubing or added to the systemic pump reservoir cause a rise of B.P. (fig. 11, *b*, *d*). In the experiment from which fig. 11, *b* is taken, the P.A.p. was not affected by the increase of B.P., and presumably the intrapulmonary communicating channels between the two vascular systems were closed down. In Experiment 45 (fig. 11, *d*), however, the B.P. rise to adrenaline injection in the systemic circulation was followed by a primary P.A.p. rise approximately 5 seconds later, and a smaller secondary rise after a further interval of 40 seconds. There seems no doubt that the primary rise of P.A.p. was due to the extra amount of blood, carrying some adrenaline with it, which was transferred from the systemic to the pulmonary circulation by way of the intrapulmonary communicating channels, and that the secondary rise of P.A.p. was in all probability due to the recirculation of adrenaline, for it coincides in time of onset with the initial flattening of the systemic B.P. recovery curve.

These results indicate the complexity of the factors which determine the pulmonary arterial pressure response to intravenous injections of adrenaline in experimental animals. It would appear that in the animal with an intact cardiovascular system, the time-relations of the adrenaline P.A.p. response depend not only upon changes in cardiac frequency and output, but upon the state of the intrapulmonary communications between the systemic and pulmonary circulations, and possibly upon the amount of adrenaline recirculating. The difficulties attending the interpretation of pulmonary vascular pressure changes due to adrenaline injections in the entire animal are therefore considerable, and are not lessened by the fact that reflexes may also play a part. We believe that entire animal perfusions will eventually do much to clarify the mechanisms involved.

Reflex Changes in Tidal Air, Systemic and Pulmonary Arterial Pressure.—The following abbreviations will be used in describing the effects of nerve stimulations: C.V.S. = cervical vagosympathetic; C.S. = cervical sympathetic; C.V. = cervical vagus; T.V.S. = thoracic vagosympathetic.

Reflex effects on the lungs were obtained in a number of experiments by stimulation of the cephalic ends of the C.V.S. nerves or of the anterior crural nerves (fig. 12). The pulmonary arterial pressure changes were frequently complicated by bronchomotor effects, and might have been due to them. In a few experiments, however, they were dissociated. A reflex rise of P.A.p. unaccompanied by a broncho-

motor response following stimulation of the cephalic ends of the C.V.S. nerves is illustrated in fig. 12, A, *a*. That the P.A.p. rise here was not due to the concomitant systemic B.P. increase transferring blood from the bronchial vascular system to the pulmonary was shown by the fact that similar B.P. alterations brought about by adjustments

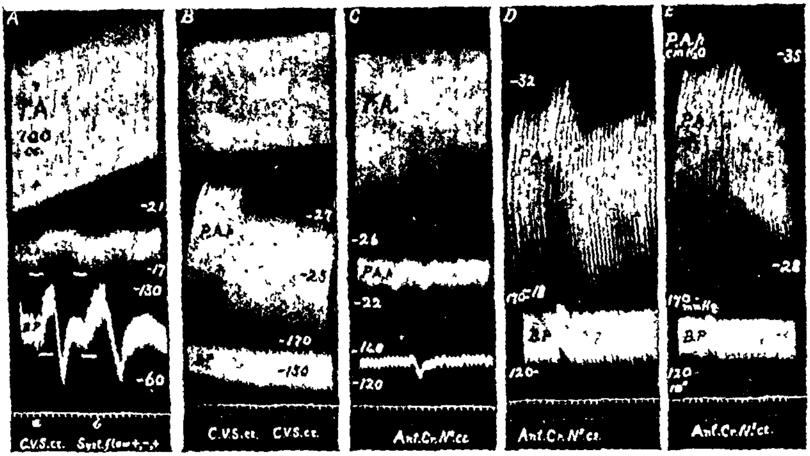

FIG. 12.—All experiments P.W.A., N.P.V. Oxygenator in circuit. Both phrenic nerves cut.

A. Expt. 44. Dog, ♂, 12·4 kg. Ether-chloralosane-cyclopropane. Both C.V.S. nerves cut; stellate ganglia and thoracic sympathetic chains intact.
a = stimulation of cephalic ends of both C.V.S. nerves. c.d. = 0 cm.
b = systemic flow adjusted to simulate B.P. changes during *a*.

B. Expt. 45. Dog, ♂, 9·6 kg. Chloralosane. Both C.V.S. cut; stellate ganglia and thoracic sympathetic chains intact. Stimulation of cephalic ends of both C.V.S. nerves at signals. c.d. = 0 cm.

C. Expt. 41. Dog, 8·7 kg. Chloralosane-cyclopropane. Both C.V.S. nerves intact; ant. crural nerve cut; lower poles of stellate ganglia separated from thoracic sympathetic chains. Stimulation of cephalic ends of ant. crural nerves. c.d. = 0 cm.

D. Expt. 47. Dog, ♂, 4·2 kg. Chloralosane-cyclopropane. Both phrenic C.V.S. and ant. crural nerves cut. Stellate ganglia and thoracic sympathetic chains intact. Stimulation of cephalic ends of ant. crural nerves. c.d. = 0 cm.

E. Expt. 47. Repetition of stimulation 24 min. after D. c.d. = 0 cm.

c.d. = coil distance.

of the systemic pump output (A, *b*) produce no immediate rise of P.A.p. The first pair of horizontal lines beneath the two blood-pressure tracings coincide with the duration of nerve stimulation; the second pair are drawn to cover that period during which the systemic arterial pressure change produced by pump adjustment simulated the systemic pressure change during nerve stimulation. It will be seen that the P.A.p. showed little or no alteration during this period. In all probability the efferent nerve pathway for the reflex P.A.p. rise was by way of the thoracic sympathetic nerves, since the C.V.S. nerves were cut.

A reflex fall of P.A.p., again with the probability that the efferent

path is by way of the thoracic sympathetic nerves, is shown in fig. 12, B. This illustrates a phenomenon we have often observed in recording either reflex P.A.p. alterations or those due to stimulation of the caudal ends of the C.V.S., C.V., or C.S. nerves. When the response was a fall of P.A.p. it frequently happened that the pressure failed to return to its initial value. This failure has also been seen, but less frequently, when the immediate P.A.p. response was a rise. Fig. 12, D and E, shows a reflex diphasic response and a fall in pressure which eventually turned out to be permanent. At this stage of the experiment the tidal air was only a few cubic centimetres, the lungs having lost most of their elasticity. A reflex bronchoconstriction without a P.A.p. change is shown in fig. 12, C. The accompanying small fall in systemic B.P. cannot account for the bronchomotor effect [Einthoven, 1892].

Tonus Variations in the Pulmonary Vascular Bed.—On several occasions stimulation of the caudal or cephalic ends of the C.V.S. nerves, and in one experiment stimulation of the cephalic ends of the anterior crural nerves, produced little or no immediate effect upon the P.A.p., but after a latent period of a minute or so marked tonus waves appeared on the P.A.p. tracing, without any change taking place in the tidal air or systemic arterial pressure. These might last as long as 10–15 minutes. An example is shown in fig. 13. This particular experiment was an extremely good one, the animal's own respiratory efforts having been quiet and regular for the first 3 hours of perfusion. Eserine (3·0 mg.) had been added to the blood 38 minutes before the observation was made, and a total of 90 μg. adrenaline in single doses of 10 or 20 μg. was also added to the blood during the previous 37 minutes. The C.V.S. nerves were cut 12 minutes previously. The size and abruptness of the P.A.p. changes seen are characteristic, and they appear to be similar to those described by Daly, Ludány, Todd, and Verney [1937] as spontaneously occurring in the perfused head-thorax preparation infused with adrenaline solution. Spontaneous variations in tonus of the pulmonary blood-vessels have been observed in isolated perfused lungs of the cat [Modrakowski, 1914; McDowall, 1921] and of the rabbit [Schafer and Lim, 1919; McDowall, 1921], but as far as we are aware they do not occur in similar lung preparations of the dog. It is interesting, therefore, that their presence in the dog has only been observed in the perfused head-thorax and perfused whole-animal preparations in which the sympathetic nervous system is intact, and/or to which adrenaline has been administered. This seems to us to be all the more significant in view of the findings of Cow [1911] that tonus waves of the isolated pulmonary artery of the rabbit occur during the contraction response to adrenaline, and of Wearn *et al.* [1926, 1928, 1934] that in the entire animal (cat) the finer blood-vessels of the lung are constantly opening and closing. These considerations lead us to speculate whether tonus waves in the pulmonary vascular

bed of the dog depend upon the integrity of the lung nerves and the concentration of adrenaline in the blood, the more so as adrenaline administration promotes variations in tone of the blood-vessels of the greater circulation in the perfused whole animal. It may be that the release of humoral substances at the pulmonary nerve terminations

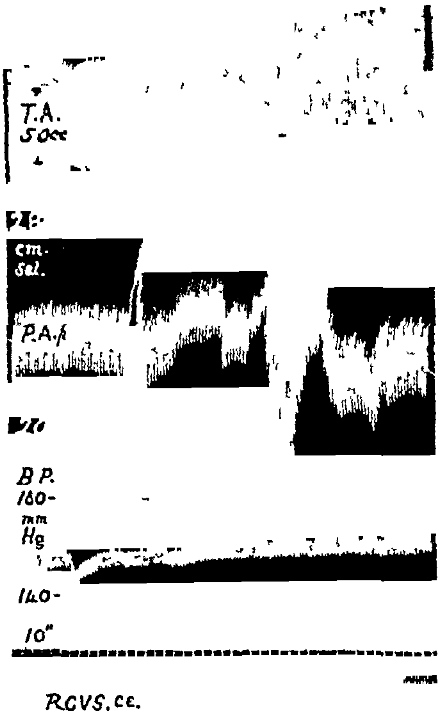

FIG. 13.—Expt. 40. Dog, ♀, 8·6 kg. Chloralosane-cyclopropane; eserine 3·0 mg. P.W.A., N.P.V. Oxygenator in circuit. Both phrenic and C.V.S. nerves cut. Stellate ganglia and thoracic sympathetic nerves intact. Stimulation of cephalic end of R.C.V S. c.d. = 12 cm. The rhythm of the respiratory efforts was recorded for a short time by signal marker at end of tracing.

during stimulation initiates tonus waves in a muscle already sensitised by adrenaline.

Stimulation of Stellate Ganglia.—In 4 experiments we confirmed that stimulation of the stellate ganglia caused a rise of P.A.p. (fig. 14). In 1 of these a slight bronchodilatation occurred. In 2 the lower pole of the stellate ganglion was separated from the sympathetic chain, so that the pulmonary vasoconstrictor fibres stimulated were passing through or relaying in the ganglion.

Pulmonary Arterial Pressure Changes due to Stimulation of the Caudal Ends of the C.V.S., C.V., and C.S. Nerves.—In many experiments

C.V.S., C.V., and C.S. stimulation caused bronchoconstriction, and we confirmed the well-known fact that this response following C.V.S. or C.V. stimulation is potentiated by eserine and suppressed by atropine. Associated with the bronchomotor effect due to C.V.S., C.V., or C.S. stimulation we frequently observed a slight rise or fall or a diphasic

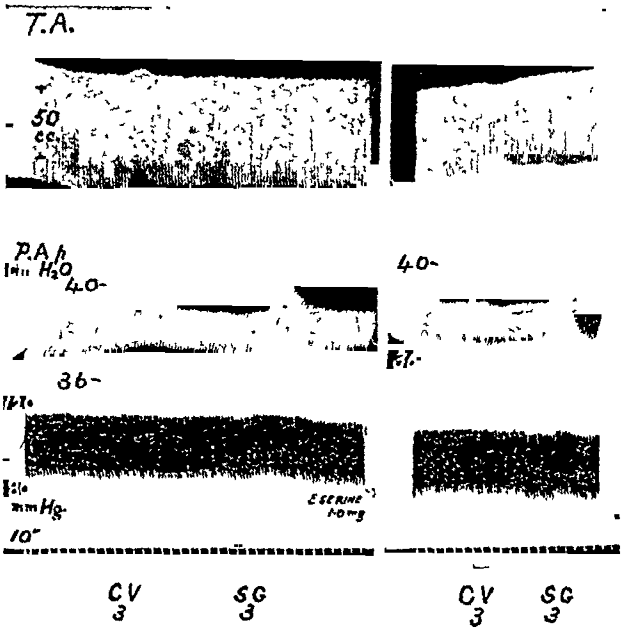

FIG. 14.—Expt. 23/21. Dog, ♀, 6·5 kg. *Cervical sympathetic nerves resected (5 cm. removed) 21 days previously.* P.W.A., N.P.V. Ether-chloralose + urethane-cyclopropane. No oxygenator Both phrenic and C.V. nerves cut; stellate ganglia isolated from thoracic sympathetic chains. Stimulation of caudal ends of C.V. nerves, c.d. = 3 cm., and of stellate ganglia, c.d. = 3 cm. before and *after eserine, 1·0 mg*

response of the P.A.p. (fig. 15). As would be expected, these responses were most easily obtained during the earlier periods of perfusion. In a few experiments, however, a slight P.A.p. rise occurred in the absence of bronchoconstriction (figs. 16 and 20, *d*). In a further attempt to obtain P.A.p. changes without the complication of bronchomotor effects we tested the action of pulmonary nerve stimulation at a time when the lungs had largely lost their elasticity. Here again stimulation of the caudal ends of the C.V.S. nerves caused a rise of P.A.p. (fig. 17). In the experiment from which these figures are taken bronchoconstriction was detectable, but the change in air entry caused thereby amounted to a few c.c. only.

The exclusion of changes in intrapulmonary pressure due to bronchoconstriction was realised in another way. After a P.A.p.

response to nerve stimulation had been obtained, the negative pressure ventilation was stopped and the stimulation repeated (fig. 18). The type of partial bronchoconstriction accompanying the P.A.p. rise in the first instance would have caused, if anything, a slight rise of P.A.p. [Daly and Hebb, 1942], but certainty that the rise could not have been due to intrapulmonary pressure changes was given by the fact that

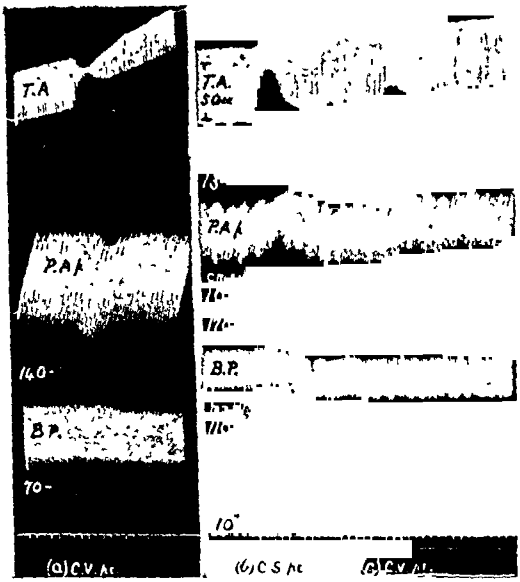

FIG. 15.—Expt. 26. Dog, ♀, 9·3 kg. P.W.A., N.P.V. No oxygenator in circuit. Ether-cyclopropane; eserine 1·0 mg. Ergotoxine 2·0 mg. Pituitrin 5·0 I.U. Phrenic nerves, stellate ganglia and thoracic sympathetic chains intact. Separated C.V. and C.S. nerves cut. Stimulation of caudal ends of both C.V. nerves. c.d. = 3 cm.

Expt. 21. Dog, ♀, 9·0 kg. P.W.A., N.P.V. No oxygenator in circuit. Ether-cyclopropane; eserine 5·0 mg. Phrenic nerves, stellate ganglia and thoracic sympathetic chains intact. Separated C.V. and C.S. nerves cut.

b = stimulation of caudal ends of both C.S. nerves, c.d. = 5 cm., and *c* of caudal ends of both C.V. nerves, c.d. = 5 cm.

the P.A.p. rose again with the second stimulation in the absence of lung ventilation.

In 2 experiments only have we observed a pure fall of P.A.p. in the absence of tidal air changes following stimulation of the caudal ends of the C.V.S. nerves. They were somewhat similar in character to the response shown in fig. 12, B, with stimulation of the central ends of the same nerves.

From these experiments we conclude that in lungs under negative pressure ventilation, stimulation of the caudal ends of the C.V.S., C.V., or C.S. nerves causes small changes of P.A.p., which can occur

independently of intrapulmonary pressure alterations which may take place as a result of concomitant bronchoconstriction.

Contractions of the Œsophagus.—A balloon was inserted into the œsophagus in 5 experiments. In none did we obtain evidence that the contractions of the œsophagus due to C.V.S. or T.V.S. stimulation affected the pulmonary arterial pressure. In 1 of these experiments,

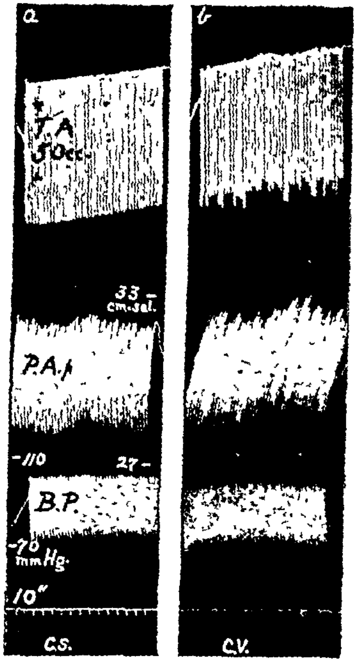

FIG. 16.—Expt. 24. Dog, 6·8 kg. P.W.A., N.P.V. No oxygenator in circuit. Ether-cyclopropane. Phrenic nerves, stellate ganglia and thoracic sympathetic chain intact. Separated C.S. and C.V. nerves cut.
a = stimulation of caudal ends of both C.S. nerves. c.d. = 6 cm.
b = stimulation of caudal ends of both C.V. nerves. c.d. = 3 cm.

made on an animal in which the left lung had been removed over three years before, L.T.V.S. stimulation caused an œsophageal contraction, no effect on the tidal air and a small fall of P.A.p. which might quite well have been due to œsophagus activity (fig. 19). A later stimulation of the R.T.V.S. caused similar effects on the œsophagus and P.A.p., and in addition a marked bronchoconstriction. A few minutes later, however, when R.T.V.S. stimulation caused no change of P.A.p. or T.A., the œsophagus showed spontaneous contractions (right-hand part of fig. 19). Since the latter had no effect on the P.A.p., it appeared unlikely that the earlier P.A.p. alterations were attributable to œsophageal contractions. These observations were made *after* 2¼ hours' perfusion, when the lung had lost its elasticity;

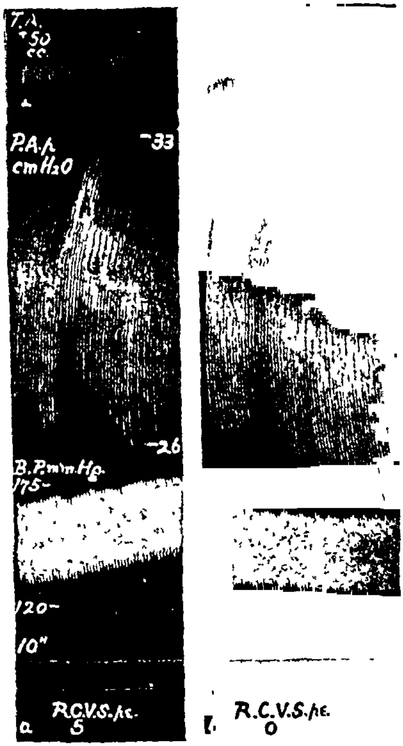

Fig. 17.—Expt. 47. Same conditions as fig. 12, D, E.
a = stimulation of caudal end of R.C.V.S. nerve. c.d. = 5 cm.
b = stimulation of caudal end of R.C.V.S. nerve. c.d. = 0 cm.

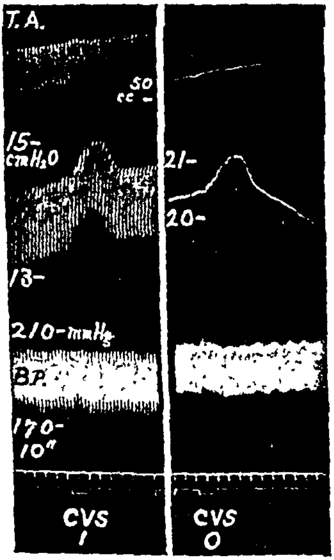

Fig. 18.—Expt. 46/31. Dog, ♀, 3·4 kg. Same conditions as in fig. 9. Stimulation of caudal ends of C.V.S. nerves with and without negative pressure ventilation. c.d. = 1 and 0 cm. respectively.

independently of intrapulmonary pressure alterations which may take place as a result of concomitant bronchoconstriction.

Contractions of the Œsophagus.—A balloon was inserted into the œsophagus in 5 experiments. In none did we obtain evidence that the contractions of the œsophagus due to C.V.S. or T.V.S. stimulation affected the pulmonary arterial pressure. In 1 of these experiments,

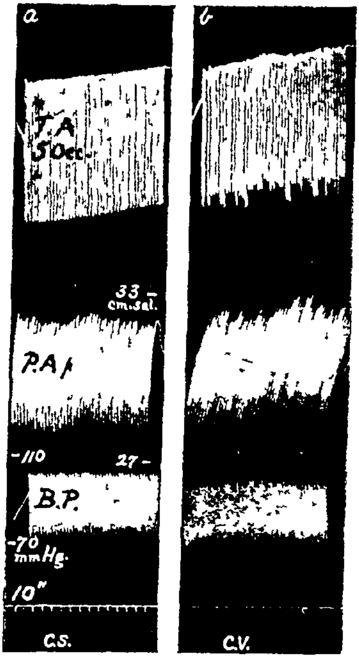

Fig. 16—Expt. 24. Dog, 6·8 kg P.W A, N.P.V. No oxygenator in circuit. Ether-cyclopropane Phrenic nerves, stellate ganglia and thoracic sympathetic chain intact. Separated C S. and C.V. nerves cut.
a = stimulation of caudal ends of both C.S nerves. c d. = 6 cm.
b = stimulation of caudal ends of both C.V. nerves c d. = 3 cm.

made on an animal in which the left lung had been removed over three years before, L.T.V.S. stimulation caused an œsophageal contraction, no effect on the tidal air and a small fall of P.A.p. which might quite well have been due to œsophagus activity (fig. 19). A later stimulation of the R.T.V.S. caused similar effects on the œsophagus and P.A.p., and in addition a marked bronchoconstriction. A few minutes later, however, when R.T.V.S. stimulation caused no change of P.A.p. or T.A., the œsophagus showed spontaneous contractions (right-hand part of fig. 19). Since the latter had no effect on the P.A.p., it appeared unlikely that the earlier P.A.p. alterations were attributable to œsophageal contractions. These observations were made after 2¼ hours' perfusion, when the lung had lost its elasticity;

of the fibres. As regards the other, the separated nerves were not tested for their effects upon the eye and heart, but, as stated earlier, we feel confident that naked-eye appearances differ sufficiently to enable them to be designated without error.

Eserine potentiated (2 expts.) and atropine suppressed (1 expt.) the bronchoconstriction due to C.S. stimulation (fig. 20). The choli-

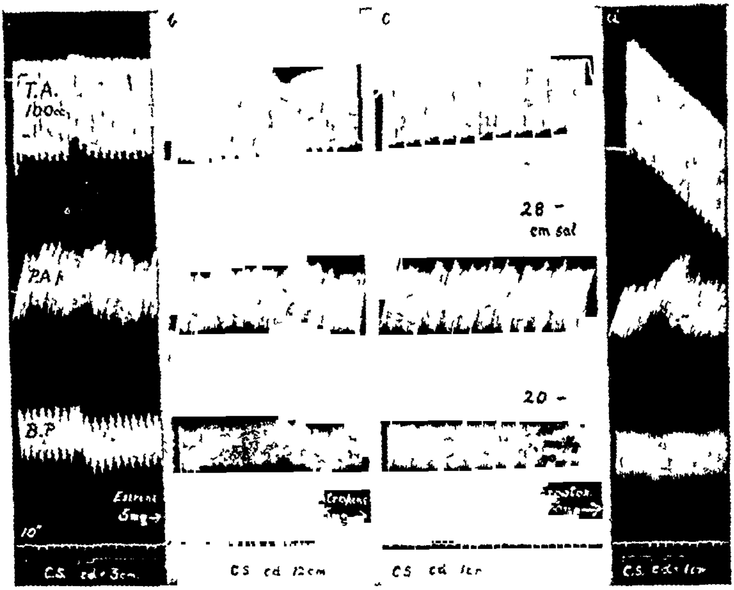

FIG. 20.—Expt. 21. Dog, ♀, 9·0 kg. P.W.A., N.P.V. Ether-chloralose + urethane-cyclopropane. No oxygenator. Phrenic nerves and separated C.V. and C.S. nerves cut. Stellate ganglia and thoracic sympathetic chains intact. C.V. and C.S. nerves tested for effect on heart and eyes respectively.

Effect of stimulating caudal ends of both C.S. nerves: (*a*) c.d. = 3 cm.; (*b*) c.d. = 12 cm.; (*c*) c.d. = 1 cm; (*d*) c.d. = 1 cm.

Eserine, 5 mg., added to pulmonary pump reservoir between (*a*) and (*b*). Atropine, 5 mg., added between (*b*) and (*c*). Ergotoxine, 7·0 mg., added between (*c*) and (*d*).

No O_2 was being admitted to closed circuit during time of tracing (*d*).

nergic fibres [Dale, 1933] involved may be preganglionic with their cell stations in the extra- or intra-pulmonary ganglia, and/or post-ganglionic terminating in the bronchial muscle. We have no evidence which would enable us to decide between these alternatives. Broncho-constriction due to C.S. nerve stimulation has also been reported by Dixon and Ransom [1912] in eserinised cats, by Saloz [1914] in pilocarpinised or eserinised animals, and by Petrovskaia [1939] in the guinea-pig, an effect which was reversed by ergotoxine.

That the bronchoconstrictor fibres in the cervical vagus may not be similar in nature or may differ in the manner by which they reach the lungs is a possibility for which we have some evidence. In 3

it was, however, the only experiment in which we suspected that a definite effect on the P.A.p. had been produced by œsophageal contractions.

Bronchomotor Changes due to C.V. and C.S. Nerve Stimulation.—In 8 experiments C.S. stimulation caused bronchoconstriction in 3 (figs. 15 and 20), no effect in 2, and a suspicion of bronchodilatation in 3. These results are somewhat similar to those of Braeucker [1926],

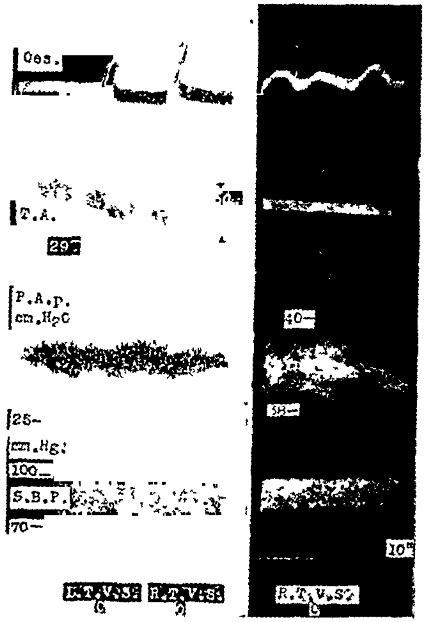

FIG. 19.—Expt. 50. Dog, ♀, 7·9 kg. *Left lung removed* 3 *years* 81 *days previously.* P.W.A., N.P.V. Ether-chloralosane-cyclopropane; eserine 4 mg. Isolated perfused lungs from another animal used in place of oxygenator. R. phrenic and both T.V.S. nerves cut. Stellate ganglia and sympathetic chains intact. Stimulation of L.T.V.S. and R.T.V.S. nerves. c.d. = 0.

who found that bronchoconstriction was produced equally well by C.S. nerve stimulation as by C.V. stimulation. He considered the possibility that fibres from the dorsal nucleus of the vagus passed caudally in the C.S. nerves. It may be that the communicating fibres between the C.V. and C.S. nerves which we met are bronchoconstrictor in function, but the fact that the bronchoconstriction was greater with C.S. than with C.V. nerve stimulation in 2 of our experiments (fig. 15, *b* and *c*) rather suggests that if this is so they are not the sole source of bronchoconstrictor fibres in the C.S. nerves. Concerning these experiments it might be asked whether any possibility arose of a mistake being made in the designation of the separated C.S. and C.V. nerves. We do not think so, for in one of these experiments the effect of stimulation upon the eye and heart was carefully tested after separation

constriction [Petrovskaia, 1939] but suppresses wholly or partly the bronchoconstriction due to stellate ganglion stimulation [Hebb, 1940]. Evidently there is a difference in the ergotoxine response between the dog and guinea-pig which demands investigation.

Effect of Stimulating the Caudal Ends of the C.V.S., C.S., and Stellate Ganglion on the Systemic Arterial Pressure.—In the perfused whole animal Dale [1928 *b*] demonstrated a small depressor effect on

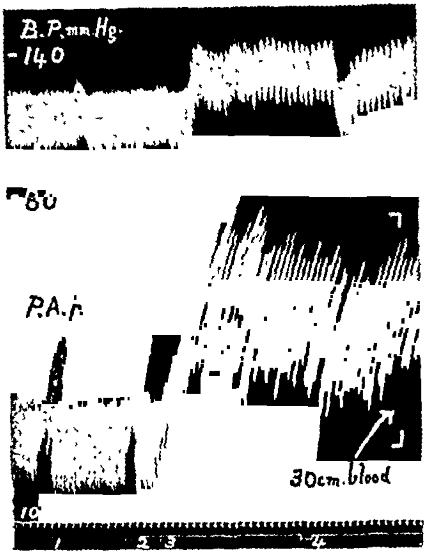

FIG. 22.—Expt. 2. Dog, ♀, 3·6 kg. P.W.A. Pernocton 1·8 c.c./kg. body-weight. No oxygenator. Phrenic and L.C.V.S. nerves, stellate ganglia and thoracic sympathetic chains intact. R.C.V.S. nerve cut. Adrenaline infusion 12·0 μg./min./kg. body-weight had been stopped 12 minutes before observation 1. Obs. 1 and 2 = stimulations of caudal end of R.C.V.S. nerve; 3 = L.C.V.S. cut; 4 = stimulation of cephalic end of R.C.V.S.

The rise of P.A.p. following section of the L.C.V.S. is probably due to an increased transfer of blood from the bronchial to the pulmonary vascular system.

stimulating the caudal end of the C.V.S. nerve, which probably represented an actual vasodilator function of the vagus for abdominal viscera. In our own experiments such stimulation produced a pressor response in 7, a depressor in 5, and a diphasic response in 1. In all cases the responses were slight. Examples of these effects are shown in figs. 10, 13, and 22. In one experiment only did we obtain definite evidence that a section of the C.V.S. nerves exerted a tonic effect (direct or reflex) on the systemic vessels (fig. 22). Excitation of the C.V. or C.S. nerves also produced only slight B.P. responses of a mixed variety (figs. 15, 20, 21). Stellate ganglia stimulation caused a slight rise in 2 out of 5 experiments. The nature of the fibres is unknown, for although eserine and ergotoxine were used as diagnostic agents in some experiments, the results were unequivocal.

experiments, in 2 of which it had already been shown that eserine potentiated the bronchoconstriction due to C.V. stimulation, the injection of ergotoxine also potentiated the bronchoconstriction due to C.V. stimulation (fig. 21). If the bronchoconstrictor fibres in the C.V. nerves are cholinergic, it seems at first sight reasonable to attribute this action of ergotoxine to its inhibiting effect on the esterase, for Loewi and Navratil [1926] and Matthes [1930] have shown that

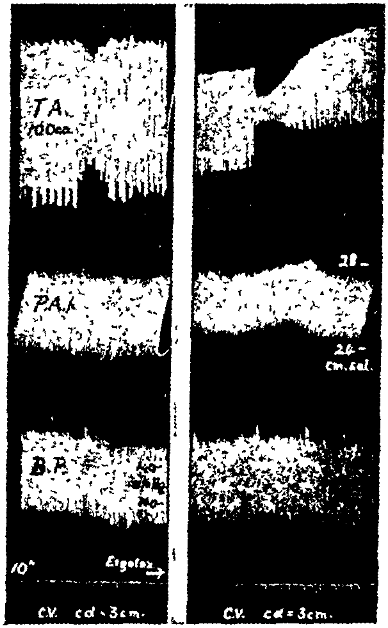

Fig. 21.—Expt. 25. Same conditions as in fig. 11, *a*. Stimulation of caudal ends of both C.V. nerves. c.d. = 3 cm. (*a*) before ergotoxine 5 mg., (*b*) after ergotoxine.

ergotamine and ergotoxine enhance the action of acetylcholine by inhibiting the action of esterase. Now we have already demonstrated that eserine potentiates the bronchoconstriction due to C.S. stimulation (fig. 20), and therefore it would be expected, if these cholinergic bronchoconstrictor fibres reach the lungs by the same route as the C.V. bronchoconstrictor fibres and are in other respects identical with them, that ergotoxine would also potentiate the bronchoconstriction due to C.S. stimulation. In point of fact, in one of our experiments in which eserine potentiated the bronchoconstriction due to C.V. and to C.S. nerve stimulation, ergotoxine failed to potentiate the bronchial response to C.S. stimulation but caused a well-marked potentiation of the response to C.V. stimulation. These results are rendered still more obscure in the light of the action of ergotoxine on guinea-pig lungs. In this animal species ergotoxine not only reverses vagal broncho-

occur when contractions fail to be detected, and on the other such contractions were seen to occur spontaneously without producing any effect on the pulmonary arterial pressure. It would, however, be advisable to eliminate the effect of contractions of this kind in every future experiment. There remains the possibility that changes of P.A.p. due to nerve stimulation may, in some of our experiments, be due not to activity of the pulmonary vascular bed proper, but to alterations in calibre of the vessels of the bronchial vascular system controlling the transfer of blood from the bronchial to the pulmonary vascular system. Thus it might be that the rise of P.A.p. following nerve stimulation we observed in some of the experiments is due to the activation of vasodilator fibres supplying the bronchial arteries, the communicating channels between the bronchial and pulmonary systems, or the bronchopulmonary veins; so that our experiments on the perfused living animal do not enable us to decide whether the pulmonary arterial responses following pulmonary nerve stimulation are due to activation of nerve fibres supplying the pulmonary vascular bed proper or of those supplying some part of the bronchial vascular system. This latter mechanism could not, of course, arise in isolated perfused lungs in which additional perfusion of the bronchial vascular system is not practised, and in which weak pulmonary vasomotor responses to nerve stimulation have been demonstrated (evidence cited in the Introduction). Thus if we interpret our present experiments in the light of the results obtained earlier on isolated perfused lungs, we are led to believe that they do demonstrate the existence of pulmonary vasomotor nerve control. For the reasons given, however, we cannot assert that our thesis is correct on the basis of the evidence offered; rather is this evidence valuable as an analysis of the difficulties which beset the interpretation of results of an investigation such as we have undertaken, and as an indication of how the problem can be more successfully attacked in the future.

Summary.

1. A description is given of a method for blood perfusion of the entire living animal (dog) in which—

(*a*) the heart ventricles are put out of action and the right auricle continues to beat and respond to vagal stimulation, its blood-supply being intact;

(*b*) the systemic and pulmonary blood-flows, the gaseous content of the blood and the ventilation of the lungs are under separate control;

(*c*) the respiratory control remains active during 3 hours' perfusion and the blood gases are maintained within normal limits;

Discussion.

We have described attempts to perfuse the whole animal under conditions which it was thought would give unequivocal evidence of pulmonary vasomotor nerve activity. We believe that the conditions laid down in the introduction to this paper for the production of such evidence were finally fulfilled; but the majority of perfused preparations were, as regards vasomotor responses, no more sensitive to pulmonary nerve stimulation than isolated lungs in which the bronchial and pulmonary vascular systems were perfused simultaneously. In only about 15 out of 40 experiments did we consider that the animal was in a really good experimental condition, yet in the vast majority of the 40 experiments bronchomotor effects due to pulmonary nerve stimulation were obtained. In 6 of these we felt reasonably satisfied that changes in P.A.p. following nerve stimulation were not due to intrapulmonary pressure changes brought about by bronchoconstriction. Poor oxygenation of the blood at least during some period of the experiment, and failure to maintain the blood CO_2 within normal limits, were in our opinion responsible for the poor condition of some of our preparations. These difficulties were finally overcome, and when the opportunity arises to continue the investigation we have every reason to expect a high proportion of successes.

In spite of the apparently good condition of some of the preparations, stimulation of the central end of a sensory nerve did not produce effects upon the systemic arterial pressure in any way comparable with those usually observed in the simply anæsthetised dog. It may be significant that some of our preparations infused with small quantities of adrenaline appeared to be more sensitive to nerve stimulation than those without administered adrenaline. However this may be, it is evident that attention will have to be concentrated upon the conditions responsible for such insensitivity and their elimination attempted.

The fact that the blood-flow through both circulations is under control and not influenced by cardiac output should prove invaluable in elucidating the mechanisms governing the control of the vascular bed of both the greater and lesser circulations. With regard to pulmonary vasomotor activity, we have confirmed the rise of P.A.p. due to stellate ganglion stimulation and have obtained weak P.A.p. responses to electrical excitation of the caudal ends of the C.V.S., C.V., and C.S. nerves—a pressure rise predominating. How far these responses are vasomotor in character or due to concomitant extra- or intra-pulmonary events requires further consideration. That they are not always due to changes of intrapulmonary pressure consequent upon the accompanying bronchoconstriction we feel reasonably certain, for they appear in the absence of tidal air changes. It also seems unlikely that they are due to contractions of the œsophagus, since on the one hand they

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(*d*) vascular and bronchomotor responses to sensory nerve stimulations and drug injections are readily obtained;

(*e*) the blood-flows and pH values of the blood are rather lower than the corresponding values given by other workers for normal unanæsthetised dogs.

2. Stimulation of the caudal ends of the cervical vagosympathetic nerves causes changes of pulmonary arterial pressure which cannot be wholly accounted for by concomitant œsophageal contractions or by bronchomotor effects. The significance of these results in relation to pulmonary vasomotor nerve activity is discussed.

3. The bronchoconstriction due to excitation of the caudal end of the cervical vagus is potentiated by eserine and suppressed by atropine, and is also potentiated by ergotoxine in the non-atropinised preparation.

We wish to express our thanks to the Government Grants Committee of the Royal Society for defraying the expenses of the investigation by a grant to one of us (I. de B. D.).

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PROLONGATION OF SURVIVAL PERIOD OF DIPHTHERIA TOXIN-INJECTED RABBITS BY CHOLESTEROL ADMINISTRATION.

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YI AND MENG (1941) found that a change in the antihemolytic strength of the plasma is correlated with a change of its total cholesterol content, and that rabbits with plasma of a higher antihemolytic strength usually survived a longer period after injection of broth culture of staphylococcus aureus. Hewitt and Todd [1939] showed that cholesterol neutralises both the hemolytic and the lethal effect of streptolysin "O" produced by hemolytic streptococci. Chassin and Bruger [1939] claimed that the antibody response to typhoid vaccine is higher in the induced cholesterolaemic rabbits. These findings led to the present investigation with the purpose of seeing whether the animals given a M.L.D. of a toxin can be brought to survive by cholesterol administration. Diphtheria toxin was selected for the present experiment.

METHODS.

Adult healthy rabbits weighing 1–2 kg. were used in all experiments. They were fed with our laboratory diet at least one week before the commencement of experimentation. The diet consisted of whole wheat, ground corn, bran, and green vegetables. Cholesterol was injected or fed daily for at least four days before the injection of diphtheria toxin. The toxin was supplied by the National Epidemic Prevention Bureau, labelled M.L.D.$=0\cdot002$ c.c. and L+ $=0\cdot18$ c.c., for which we wish to express our sincere thanks to Dr. F. F. Tang, Director of the Bureau. The dosage of toxin we adopted was 0·002 ml. per 250 g. body-weight. It was given subcutaneously in all the experiments. Ten per cent. cholesterol in vegetable oil was administered subcutaneously. Sodium chloride was given by mouth, intravenously, or intraperitoneally in 1 per cent. solution and cholesterol + lecithin and lecithin were given in a 5 per cent. emulsion in vegetable oil-water (1 : 1). Aseptic precautions were observed in all injections and blood samplings (from marginal ear vein).

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TABLE II.—SURVIVAL PERIODS AFTER CHOLESTEROL AND DIPHTHERIA TOXIN.

	Oil.	Chol-esterol inj.	Chol-esterol fed.	Diph. toxin.	Toxin and chol. inj.		Toxin and chol. feeding.	Toxin and NaCl and chol.	Toxin and NaCl.	Toxin and lecithin.	Toxin and lecithin and chol.	
					A *	B *					A †	B †
No. of rabbits	6	6	3	16	6	11	12	9	9	4	5	5
Average survival period, days . .	..	..	..	56 hr.	6	16	8	8	70 hr.	75 hr.	58 hr.	74 hr.
No. survived	all	all	all	0	0	2	1	1	0	0	0	0

A * Cholesterol injection stopped 4 or 5 days after toxin administration.
B * Cholesterol injection continued until death.
A † Daily injection of 100 mg. cholesterol and lecithin each.
B † Daily injection of 200–400 mg. cholesterol and 100 mg. lecithin.

injections) to reach a maximum and about 7–10 days to return to normal level after cessation of injection.

3. *Feeding of Cholesterol.*—The slowness of subcutaneous absorption has led us to try feeding. In this group the same amount of cholesterol was given with a mixture of whole wheat and corn-flour which were made to a paste with water. All of these animals were normal and survived. The results are also shown in Tables I. and II. The elevation of their total plasma cholesterol content was, however, greater than after injection.

4. *Diphtheria Toxin Injection.*—In preliminary control experiments we have found that 0·002 ml. of the toxin per 250 g. body-weight was the M.L.D. which caused death within 44–92 hours. Hence in all subsequent experiments we have adopted this dosage with 2–4 days survival period as the standards.

In this control group the total cholesterol content of the plasma is higher than normal (Table I.); whereas that of suprarenals and to a lesser degree of the liver was lower (Table III.). It is quite possible that the elevation of plasma cholesterol may come about at the expense of the tissue cholesterol. What significance this may have we do not know.

TABLE III.—TOTAL CHOLESTEROL CONTENT OF VARIOUS TISSUES (in mg. p.c. wet weight).

Tissues.	Normal.	Cholesterol injected or fed.	Diphtheria toxin injected.	Toxin + cholesterol injected.
No. of rabbits .	4	6	4	4
Heart . . .	118	160	120	145
Liver . . .	308	492	282	430
Suprarenals . .	5632	7325	2630	4745

Plasma and tissue total cholesterol were determined according to the method of Bloor (described in Peters and Van Slyke's *Clinical Chemistry*). They were estimated in a number of animals 2 or 3 times before, and at frequent intervals after, the administration of oil, cholesterol, toxin, etc. The tissues from adrenals, liver, and heart of various groups of animals were obtained just after death. In a few cases free and combined cholesterols were determined according to the modified method of Schoenheimer and Sperry [1934].

In order to see whether the animals became anæmic or not, we have occasionally made cell volume and hemoglobin estimations, but found no change in these.

A few tissues were fixed in Bouin's fluid and sectioned with ordinary histological technique in order to examine the pathological changes brought about by the toxin.

Results.

Control Experiments.

1. *Oil Injection.*—As a control group rabbits were given daily injections of 2–4 ml. sterile vegetable oil and their plasma cholesterol and general health such as body-weight, temperature, etc., were carefully observed. The only change that could be detected was a slight elevation of total cholesterol content of the plasma (Table I.). They were all in good condition and none died up to the end of the experiment, which usually lasted over two months.

Table I.—Total Cholesterol Content of the Plasma.

	Normal.	Oil inj.	Chol. inj.	Chol. fed.	Diph. toxin inj.	Toxin + chol. inj.	Toxin + chol. fed.
No. of determinations	88	11	17	8	8	15	8
Average cholesterol conc. mg./100 ml.	53 (30–66)	69 (56–72)	116 (98–130)	244 (210–290)	109 (74–151)	189 (147–210)	314 (240–390)

2. *Injection of Cholesterol in Oil.*—Another control group of 6 rabbits were injected with 200–400 mg. cholesterol in oil 5 times, once every other day. In these animals we could see no abnormality except an elevation of total cholesterol content of the plasma (Table I.). They are still alive (Table II.) and active up to the end of the experiment.

It may be remarked that the rise of plasma cholesterol following subcutaneous administration is rather slow and the return to normal level occurs also slowly; it takes several days (usually after three

The Effect of Sodium Chloride Administration.

According to our own observation the clinical manifestation of diphtheria toxin-injected rabbits appears similar to suprarenal insufficiency. We wish we could try cortical hormone preparations, but the present situation has forbidden us to do so. Nevertheless, we have tried sodium chloride administration, with the object of seeing whether the symptoms of intoxication can be ameliorated and life prolonged. Two groups of rabbits were used, one being given toxin and sodium chloride (100–600 mg. daily) in addition to that already present in the diet, and the other toxin, sodium chloride, and cholesterol. In the first group none of the 9 animals survived longer than 102 hours, with an average of only 70 hours (Table II.). In the second group 2 out of 9 animals died in 66 and 68 hours respectively, while 6 survived from 5 to 13 days, with an average of 8 days, and 1 is still living (Table II.). From this finding it is obvious that with the amount of sodium chloride we administered no beneficial effect can be obtained.

The Effect of Lecithin.

Since the metabolism of cholesterol is known to be closely connected with that of lecithin, we have also tried lecithin. Here we used also two groups of rabbits, one group given lecithin alone and the other lecithin and cholesterol.

Lecithin was given subcutaneously in the form of emulsion equivalent to 100 mg. daily before and after diphtheria toxin injection. The animals all died within the range of time found in the control group (Table II.).

In the other group 5 per cent. lecithin + cholesterol emulsion in 1 : 1 oil and water given subcutaneously daily (100 mg. of cholesterol and lecithin each) before and after diphtheria toxin. The animals survived only 46–84 hours, with an average of 58 hours (Table II.).

The absence of beneficial effect of lecithin + cholesterol in the latter group may be due to a harmful influence of the lecithin or to an insufficient quantity of cholesterol being given. To test which of these factors was in operation we have made another series of experiments in which the amount of cholesterol injected together with lecithin was increased to 200–400 mg. daily. It was found that all rabbits used in this group died within 81 hours, with an average of 74 hours (Table II.). Since they would have survived much longer if they were given the same amount of cholesterol without lecithin, we conclude that lecithin abolishes or diminishes the beneficial effect of cholesterol.

The most definite clinical symptoms of diphtheria toxin attack in rabbits were anorexia, loss of body-weight, initial brief rise of temperature followed by a profound fall at the fatal stage, weakness and, in most cases, diarrhœa. They were similar to those in suprarenal insufficiency. Gross autopsy just after death showed in many cases congestion and hæmorrhage in the intestines, liver, kidneys, testicles, spleen, and sometimes the lungs, ulceration of stomach and sometimes of small intestines. But the most marked changes appeared in the suprarenal glands which showed congestion, hæmorrhage, and enlargement; in some cases necrosis and cavitation were found. The gross findings were verified in several cases by microscopic examinations.

Injection of Diphtheria Toxin and Cholesterol.

After feeding for one week or more on laboratory diet, the animals were injected with 200–400 mg. cholesterol in oil daily, for at least 4 days. Diphtheria toxin was then injected, while cholesterol administration was continued for 4 days in one group (A), and until death in another (B) (Table II.). In the former group the animals survived 5–9 days, with an average of about 6 days, and in the latter group 2 out of 11 animals lived indefinitely, while the remaining 9 rabbits survived 9–25 days, with an average of 16 days. If we put all the animals which died of these two groups together, we see their survival period extended from 5–25 days, with an average of 12 days, which is much longer than that of the control group. This finding leads to the suggestion that prolonged cholesterol administration is beneficial.

In this series of experiments the plasma cholesterol level of all animals remained high throughout the survival period. On the other hand, if the animals died within a week or so after toxin administration, the cholesterol content (in mg. per 100 g. wet weight) of the suprarenals was found slightly below the normal level (column 5 of Table III.), though still higher than those that died of toxin without cholesterol administration. The change of free cholesterol and its ratio to esterified and total cholesterol is not consistent. Our meagre data do not justify any definite conclusion.

Regarding the autopsy finding it varies with different animals, but in general, if the animals died within a week or so, lesions were broadly similar to those of controls. On the other hand, if they died one week or more after toxin injection, gross lesion was almost undetectable.

When cholesterol was fed daily until death instead of being injected, the animals survived for a slightly shorter time, though still much longer than the controls. Of 12 animals, 11 survived 5–10 days, with an average of 8 days, 1 being alive up to the termination of the experiment. Other findings are similar to those of the cholesterol-injected group.

plasma cholesterol. Is the depreciation of suprarenal cholesterol the cause of death? We have no evidence of it. Administration of cholesterol may improve the condition, but the question is, how can we make the suprarenals and other tissues utilize it?

Table IV.—Wet Weight of One Suprarenal Gland.

	Normal.		Toxin-injected (died).		Toxin + cholesterol-injected (survived).	
	Body-weight, kg.	Wet weight of suprarenal, mg.	Body-weight, kg.	Wet weight of suprarenal, mg.	Body-weight, kg.	Wet weight of suprarenal, mg.
	1·220	49	0·950	88	1·280	56
	1·310	52	1·080	94		
	0·950	74	1·200	137		
			1·210	154		
Average	1·130	58	1·110	118	1·280	56

Summary.

1. Several groups of rabbits were given cholesterol, lecithin, cholesterol + lecithin, sodium chloride or sodium chloride + cholesterol respectively, before and after the injection of a M.L.D. of diphtheria toxin. The symptoms and survival period of these animals were recorded.

2. Cholesterol definitely improves the conditions and prolongs the life of the toxin-injected animals. In 38 rabbits given cholesterol or cholesterol + sodium chloride, 10·5 per cent. lived indefinitely, 84·2 per cent. survived 5–25 days, with an average of about 9 days, and 5·2 per cent. died within the period of 66–68 hours, while control animals with diphtheria-toxin injection alone all died within 44–92 hours.

3. Sodium chloride and lecithin respectively cannot prolong the life of the toxin-injected rabbits any longer than that of the control group. Lecithin appears to abolish the beneficial effect of cholesterol.

4. The suprarenal glands of the diphtheria toxin-injected rabbits are enlarged, but the *total* cholesterol concentration is reduced. This reduction is probably due to the increment of the tissue mass without a corresponding addition of cholesterol.

Discussion.

Among the 38 rabbits in three series of experiments with diphtheria toxin and cholesterol treatments with and without the addition of sodium chloride, 4 of them or 10·5 per cent. survived indefinitely, 2 of them or 5·2 per cent. died within 66 and 68 hours, and the remaining 32 rabbits or 84·2 per cent. survived 5–25 days (on an average 9 days). Since control animals injected with diphtheria toxin alone all died within 44–92 hours, we may conclude that cholesterol administration prolongs the life of diphtheria toxin-injected rabbits, and in a few cases prevents death from the effect of the toxin.

On the other hand, lecithin injection is useless or even harmful. When it was given with cholesterol, all animals died within 84 hours. Apparently lecithin abolishes thebeneficial effect of cholesterol.

Many investigators have reported that the survival period of adrenalectomized animals can be prolonged by excessive sodium chloride administration [Clark and Barnes, 1940; Anderson *et al.*, 1940; and others]. Although as a result of diphtheria toxin we have seen severe pathological changes in the suprarenal glands, we have failed to ameliorate the symptoms and prolong the life of toxin-injected animals by giving sodium chloride alone. It is quite possible that the dosage of sodium chloride given was too small. But since it had not the slightest beneficial effect, we do not think it likely that any marked improvement would be effected by giving larger dosages. This, however, does not rule out the possibility that the death of diphtheria intoxication is associated with suprarenal insufficiency. Further work must be done in order to clarify this point.

That the high plasma cholesterol level is not essential for improvement of the condition and for prolongation of the life of diphtheria toxin-injected animals is shown by the elevation of plasma cholesterol concentration in all moribund animals, with or without previous administration of cholesterol.

The question as to how cholesterol exerts its beneficial effect can only be conjectured at the present time. Since cholesterol is an essential cell constituent, and since it is known to protect the red blood cells from the attack of various chemical lysins, there is a possibility that it may also protect other cells by its ability to neutralize toxins or to prevent the penetration of toxins into the tissue cells. It is observed that when the animal is at the fatal stage of toxin attack, or shortly after death, the cholesterol content of the suprarenals falls considerably. This drop of cholesterol concentration is not due to the increase of water content which we have found in several cases that died of diphtheria intoxication, but is due to the enlargement of the glands without a corresponding increase of total cholesterol (see Tables III. and IV.). Apparently, the suprarenals fail to utilize the

THE EFFECT OF TEMPERATURE ON THE ANTIHÆMOLYTIC ACTIVITY OF LECITHIN AND CHOLESTEROL. By

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If the interaction between hæmolysin and antihæmolysin is purely of a chemical nature and is independent of the activity of the cells, we should expect to observe an acceleration of the reaction by temperature. So far as we can ascertain from the available literature very little work has been done regarding this point. A series of experiments was therefore carried out in which the hæmolysin-antihæmolysin systems were subjected to different temperatures for a definite length of time and then cooled to room temperature. This was followed by adding cell suspension. After a thorough shaking the mixture was placed in a water-bath at 37° C. for 30 minutes and the degree of hæmolysis then determined, as described in the preceding paper. The hæmolysin-antihæmolysin systems were made up in exactly the same way as before and the hæmolytic or antihæmolytic potency of the mixture was calculated in terms of micrograms of the lysin in the 3-ml. system required to produce 50 per cent. hæmolysis or inactivation. The detail of procedure and computation has already been described in the preceding communication.

The temperature quotient or Q_{10} was obtained by the following equation:—

$$Q_{10}=\frac{\text{hæmolysin inactivated at } t_2}{\text{hæmolysin inactivated at } t_1},$$

where t_2 represents the temperature 10 degrees above t_1.

Results.

For the sake of brevity it is necessary to omit some of our numerical data, especially concerning negative findings, and present only the essential points.

[1] Fellow of British Boxer Indemnity Fund Administration.

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when the velocity of reaction is at its peak. These quotients (Q_{10} of 2·2–4·0) signify the chemical nature of saponin-cholesterol reaction.

(b) *By Lecithin.*—Lecithin is practically thermostable in so far as its hæmolytic activity is concerned, because its hæmolytic potency is not weakened even after being heated to 98° C. for 30 minutes. This is shown in Table I. When lecithin and cholesterol are present

TABLE I.—THE HÆMOLYTIC POTENCY OF LECITHIN AFTER HIGH TEMPERATURE TREATMENT.

	Lecithin required for 50 per cent. hæmolysis, μg.
Unheated sample	1800
Heated sample (98° C. for 30 minutes)	1800

together without other lytic agent, lecithin is hæmolytic, and its action may be inhibited by cholesterol. The interaction between these two substances is not accelerated by increasing the temperature as high as up to 98° C. for 30 minutes. Table II. records the results of a typical experiment of this series which justifies our statement made above.

TABLE II.—THE EFFECT OF TEMPERATURE ON THE ANTI-LECITHIN ACTION OF CHOLESTEROL.

	Incubation temperature, degrees C.	Incubation period, minutes.	Lecithin inactivated, μg.
Lecithin + cholesterol (0·025 mg.)	20	10	60
,, ,, ,,	38	30	60
,, ,, ,,	98	30	60

If lecithin is mixed with other hæmolysins, it may remain hæmolytic or become antihæmolytic. The effect of temperature on the reactions between lecithin and other lysins will be reported subsequently. However, we feel it pertinent to mention here its reactions in the saponin system. The inactivation of saponin by lecithin is readily completed at 20° C.; no further inactivation was demonstrable in higher temperatures. Table III. verifies this statement.

(c) *By Cholesterol + Lecithin.*—The combined antihæmolytic action of cholesterol and lecithin against saponin hæmolysis is accelerated by subjecting the system to higher temperatures above 20° C. As shown in fig. 2, the saponin (in μg.) inactivated at 20° C. is much less

Inactivation of Saponin and Digitonin.

(a) *By Cholesterol.*—While subjecting saponin and cholesterol to different temperatures separately before adding cells does not cause any alteration in their respective hæmolytic and antihæmolytic behaviour, similar treatment of the mixture of these two substances gives rise to a remarkable change in the resultant antihæmolytic potency. This is shown by the graphs in fig. 1. There are five curves

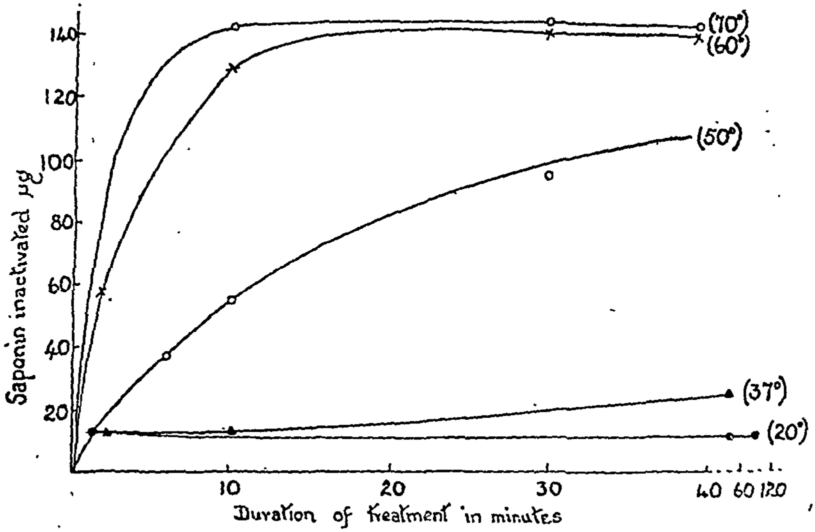

Fig. 1.—The influence of temperature on cholesterol activity against saponin hæmolysis.

from five different temperature treatments. It is noted that the inactivation of saponin by cholesterol increases with temperature, and that at a given temperature the rate of increase is most rapid during the first few minutes of treatment; below 37° C. acceleration stops abruptly at the first or second minute, whereas with higher temperatures the curves continue to rise, though at a slower rate, up to 10, 30, and 60 minutes respectively for 70°, 60°, and 50° C.

Careful analysis of these curves indicates that there seem to be two processes operating in the inactivation of saponin by cholesterol, one being present at all temperatures above 20° C. and the other accelerated only by high temperatures above 40° C. Corroborative evidence has been obtained when lecithin was added to the system. Lecithin appears to accelerate the latter process at a lower temperature.

From the graphs in fig. 1 we have worked out the Q_{10}. The amount of saponin in micrograms inactivated by cholesterol at 60° C. for 10 minutes is 2·2 times greater than at 50° C., and at 48° C. about 4 times that at 38° C. These values, of course, apply only to the period

when the velocity of reaction is at its peak. These quotients (Q_{10} of 2·2–4·0) signify the chemical nature of saponin-cholesterol reaction.

(b) *By Lecithin.*—Lecithin is practically thermostable in so far as its hæmolytic activity is concerned, because its hæmolytic potency is not weakened even after being heated to 98° C. for 30 minutes. This is shown in Table I. When lecithin and cholesterol are present

TABLE I.—THE HÆMOLYTIC POTENCY OF LECITHIN AFTER HIGH TEMPERATURE TREATMENT.

	Lecithin required for 50 per cent. hæmolysis, μg.
Unheated sample	1800
Heated sample (98° C. for 30 minutes)	1800

together without other lytic agent, lecithin is hæmolytic, and its action may be inhibited by cholesterol. The interaction between these two substances is not accelerated by increasing the temperature as high as up to 98° C. for 30 minutes. Table II. records the results of a typical experiment of this series which justifies our statement made above.

TABLE II.—THE EFFECT OF TEMPERATURE ON THE ANTI-LECITHIN ACTION OF CHOLESTEROL.

	Incubation temperature, degrees C.	Incubation period, minutes.	Lecithin inactivated, μg.
Lecithin + cholesterol (0·025 mg.)	20	10	60
,, ,, ,,	38	30	60
,, ,, ,,	98	30	60

If lecithin is mixed with other hæmolysins, it may remain hæmolytic or become antihæmolytic. The effect of temperature on the reactions between lecithin and other lysins will be reported subsequently. However, we feel it pertinent to mention here its reactions in the saponin system. The inactivation of saponin by lecithin is readily completed at 20° C.; no further inactivation was demonstrable in higher temperatures. Table III. verifies this statement.

(c) *By Cholesterol + Lecithin.*—The combined antihæmolytic action of cholesterol and lecithin against saponin hæmolysis is accelerated by subjecting the system to higher temperatures above 20° C. As shown in fig. 2, the saponin (in μg.) inactivated at 20° C. is much less

Table III.—The Effect of Temperature on the Anti-Saponin Action of Lecithin.

	Incubation temperature, degrees C.	Incubation period, minutes.	Saponin inactivated, μg.
Lecithin + saponin . . .	20	20	5
,, ,, . . .	50	20	5
,, ,, . . .	60	20	5

than that at 38° C. However, at 50° C. the amount of saponin inactivated is only slightly higher than that at 38° C. It appears that the rate at 38° C. approaches almost the maximum. During the

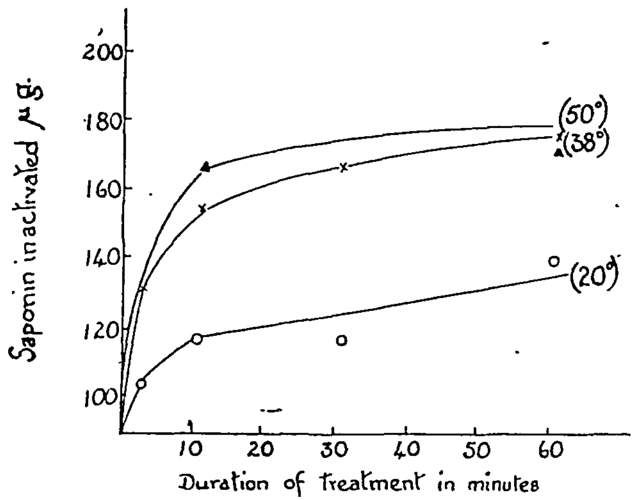

Fig. 2.—*The influence of temperature on the combined action of lecithin and cholesterol toward saponin hæmolysis.*

most rapid period of inactivation, that is within the first 10 minutes, the Q_{10} is 0·89 for 38° to 48° and 0·71 for 20° to 30° C.

Another interesting point is that the process of inactivation proceeds steadily even at 20° C., which would not occur if lecithin is removed from the system. A comparison of these curves with those in fig. 1 at once suggests that the reinforcing effect of lecithin on the anti-hæmolytic action of cholesterol may be chiefly catalytic in nature.

Digitonin behaves similarly to saponin as regards temperature effect. To avoid repetition of statement the results of a typical experiment summarized in Table IV. suffice to illustrate our essential findings.

TABLE IV.—THE EFFECT OF TEMPERATURE ON THE ANTI-DIGITONIN ACTION OF CHOLESTEROL WITH AND WITHOUT LECITHIN.

	Incubation temperature, degrees C.	Incubation period, minutes.	Digitonin inactivated, μg.
Digitonin + cholesterol . .	27	10	25
,, ,, . .	37	10	110
,, ,, . .	47	10	146
,, ,, . .	57	10	152
,, ,, . .	98	10	162
Digitonin + cholesterol + lecithin .	27	10	200
,, ,, ,, .	37	10	200
,, ,, ,, .	47	10	222
,, ,, ,, .	57	10	222
,, ,, ,, .	98	10	222

Inactivation of Crude Bile Acid.

(a) *By Cholesterol.*—The hæmolytic potency of crude bile acid remains unchanged even when heated to 50° or 90° C. for 30 minutes. The interaction between cholesterol and crude bile acid is accelerated at a higher temperature. As shown in fig. 3, the process of inactivation

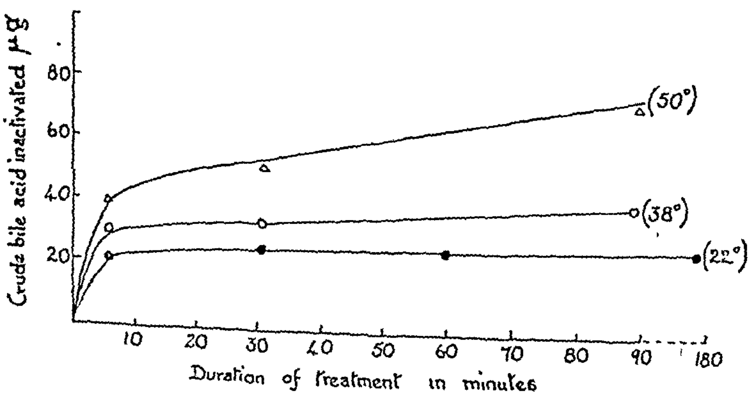

FIG. 3.—The influence of temperature upon the activity of cholesterol against crude bile acid hæmolysis.

at 22° C. stops abruptly within the first 10 minutes. At 38° or 50° C. the amount of crude bile acid inactivated gradually increases as time of incubation becomes longer. The curves representing the relation of the amount of bile acid inactivated to time of incubation at three temperatures take a shape similar to those obtained from the interaction between saponin and cholesterol. However, the Q_{10} in the

former case is much lower. Thus, for instance, it is only 1·1 from 38° to 48° C. when the incubation period is 30 minutes.

(b) *By Lecithin.*—Lecithin is antihæmolytic against crude bile acid. The interaction between these two substances takes place and is completed rapidly, even at room temperature. Increasing the incubation temperature does not help in quickening the rate of the reaction.

(c) *By Cholesterol + Lecithin.*—When cholesterol and lecithin are both present in the crude bile acid system the reinforcement of antihæmolytic action takes place very quickly and reaches a maximum within a relatively short period of time even at a low temperature, *i.e.* subjection to higher temperatures does not accelerate the rate of inactivation.

Inactivation of Natural Hæmolysin.

Since the complement of natural hæmolytic serum or plasma is rapidly destroyed above 50° C., we can only study the effect of temperature below this point. The experiments we have thus performed are as follows: one sample of cholesterol + natural hæmolysin was kept at 37° C. for 10, 60, or 90 minutes, and another sample at 25° to 29° C. for the same length of time. They were then brought to room temperature before adding cells. Similar procedure was applied to the studies of lecithin and lecithin + cholesterol. The results of one series of such experiments are presented in Table V. It is seen that

TABLE V.—THE EFFECT OF TEMPERATURE ON THE ANTIHÆMOLYTIC ACTION OF CHOLESTEROL AND LECITHIN AGAINST HÆMOLYTIC SERUM.

	Incubation temperature, degrees C.	Incubation period, minutes.	Hæmolytic serum inactivated, μl.
Serum alone	25	90	0
,, ,,	37	90	0
Serum + lecithin (0·8 mg.)	25	90	80
,, ,, ,,	37	90	80
Serum + cholesterol (0·2 mg.)	25	90	110
,, ,, ,,	37	90	130
Serum + cholesterol + lecithin	25	90	90
,, ,, ,,	37	90	100

in the serum the antihæmolytic action of lecithin is not affected by temperature, whereas that of cholesterol and cholesterol + lecithin is accelerated, the acceleration in the latter system being less marked. In this particular experiment one notes the absence of temperature effect (within the range used) upon the hæmolytic potency of the serum.

This is, however, not invariable, especially when citrated plasma was used in place of serum. Nevertheless, this variation does not invalidate our main conclusion, since the amount of hæmolysin inactivated by lecithin and cholesterol was always calculated by subtracting the hæmolytic potency of the serum or plasma under the same temperature.

Inactivation of Oleic Acid, Taurocholate, and Glycocholate.

The hæmolytic power of taurocholate and glycocholate is not weakened by subjecting them to boiling temperature for 15 to 30 minutes; on the other hand, that of oleic acid is remarkably decreased by temperature above 60° C. The rate of antihæmolytic action of cholesterol toward these lysins remains practically unchanged after different temperature treatments. Table VI. presents some of our data for illustration. The same is true with lecithin and lecithin + cholesterol (see also Table VI.).

Table VI.—The Effect of Temperature on the Antihæmolytic Action of Cholesterol and Lecithin against Oleic Acid, Taurocholate, and Glycocholate Hæmolysis.

	Incubation temperature, degree C.	Incubation period, minutes.	Lysins inactivated, μg.
Oleic acid + cholesterol . . .	28	30	27
" " . . .	37	30	27
" " . . .	50	30	27
Oleic acid + lecithin . . .	28	30	(−) 10
" " . . .	37	30	(−) 10
" " . . .	50	30	(−) 10
Oleic acid + cholesterol + lecithin .	28	30	22
" " " .	37	30	22
" " " .	50	30	22
Taurocholate + cholesterol . .	24	60	430
" " . .	37	60	430
" " . .	50	60	430
Taurocholate + lecithin . . .	24	60	(−) 170
" " . . .	50	60	(−) 170
Taurocholate + cholesterol + lecithin .	24	60	380
" " " .	50	60	380
Glycocholate + cholesterol . .	28	15	374
" " . .	50	15	434
" " . .	97	15	434
Glycocholate + lecithin . . .	28	15	(−) 186
" " . . .	50	15	(−) 186
" " . . .	97	15	(−) 206
Glycocholate + cholesterol + lecithin .	28	15	214
" " " .	37	15	234
" " " .	50	15	234
" " " .	97	15	234

Discussion.

It is well known that the rate of a chemical reaction is usually accelerated by a rise of temperature, while that of physical process may be accelerated or may not. The Q_{10} of many chemical reactions is recognized to lie between 2 and 3, but that of a physical process is usually below 2 or negative. Nevertheless, the reactions of many inorganic substances, especially ionic in nature, are so fast or instantaneous at ordinary temperature that the effect of rise of temperature can hardly be detected with ordinary laboratory technique. On the other hand, the reactions between hæmolysin and antihæmolysin we have studied in the present experiments are relatively of a slow type because of their complex organic nature, and hence the effect of temperature on the rate of their reactions can be easily observed and the Q_{10} computed.

The rate of interaction between cholesterol and saponin, digitonin, crude bile acid, and natural hæmolysin is accelerated in different degrees by a rise of temperature. As the Q_{10} values for cholesterol-saponin and cholesterol-digitonin are found to lie between 2 and 4, it is very likely that their reactions are purely chemical in nature. On the other hand, those of cholesterol-crude bile acid and cholesterol-natural hæmolysin are less than 1, and therefore the reactions involved must be physical or physico-chemical in nature.

The rate of cholesterol activity and the interaction between cholesterol and lecithin in oleic acid, Na-taurocholate, and Na-glycocholate systems is not accelerated by a rise of temperature. Similarly that between lecithin and other lytic agents employed in the present study is also apparently not affected by temperature. The absence of demonstrable temperature effect might be explained as being due to the high velocity of reaction, resembling that between many inorganic compounds; but the facts indicate the contrary, because the reactions of cholesterol and lecithin toward these hæmolytic agents are not at all instantaneous or different in speed from those between cholesterol and saponin and digitonin. We therefore propose that the changes involved in the antihæmolytic activity of cholesterol against lecithin, oleic acid, Na-taurocholate, and Na-glycocholate, and that involved in the activity of lecithin toward other lytic agents must be physical in nature.

It may be recalled that at ordinary room temperature the cholesterol-saponin, -digitonin and -crude bile acid reactions have an initial rapid rate and become very slow in the later stages. Nevertheless, in the relatively quiescent stage they may be accelerated by lecithin. This fact suggests that lecithin seems to catalyse the antihæmolytic activity of cholesterol against saponin, digitonin, and crude bile acid. Corroborative evidence in support of this hypothesis is

furnished by the effect of lecithin in diminishing or abolishing the temperature influence upon these systems. The explanation we offer is that lecithin quickens the reaction and makes it complete at ordinary room temperature within a relatively short period of time, so that no further increase of reaction rate can be demonstrated by the rise of temperature.

Another peculiar phenomenon observed in this series of experiments is the effect of temperature on the reaction of cholesterol toward crude bile acid and taurocholate or glycocholate systems. The first system is accelerated by increasing temperature, whereas the latter two systems are not. This opposite behaviour may be attributed to the impurities contained in our bile acid preparation. Since the behaviour of our bile acid preparation is in many respects similar to that of saponin and digitonin, and different from that of taurocholate and glycocholate, we are justified in postulating the existence in the bile acid preparation of some other hæmolytic substance having a constitution similar to the hæmolytic glucosides in question.

It is interesting to note that lecithin is antihæmolytic to those lysins whose reaction with cholesterol is influenced by temperature, but hæmolytic to those whose reaction with cholesterol is not affected by temperature. Is it merely a coincidence, or has it any significance? At present our data are too meagre to offer any adequate interpretation. However, if we assume that those hæmolytic and antihæmolytic reactions that are independent of temperature are physical in nature, it follows that the hæmolytic action of lecithin must also be a physical process. On the other hand, those whose reactions are accelerated by increasing temperature are chemical or physico-chemical; the antihæmolytic behaviour of lecithin must follow also the same process.

Summary.

1. The antihæmolytic reactions of cholesterol toward saponin, digitonin, crude bile acid, and natural hæmolysin are accelerated by a rise of temperature, whereas those of cholesterol toward lecithin, oleic acid, Na-taurocholate, and Na-glycocholate are not.

2. The Q_{10} of cholesterol-saponin and cholesterol-digitonin systems lies between 2 and 4 and that of cholesterol-crude bile acid or cholesterol-natural hæmolysin is less than 1, indicating that the reactions in the former group are probably chemical in nature, while those in the latter group are physical or physico-chemical.

3. The interaction between lecithin and all other hæmolysins studied are not accelerated by the rise of temperature. This is unlikely to be due to the high velocity of their reaction at low temperature.

4. The degree of temperature effect in cholesterol-saponin and cholesterol-digitonin systems is smaller with the presence of lecithin

than without. On the other hand, there is no temperature effect on the rate of combined antihæmolytic action of cholesterol and lecithin toward other hæmolysins.

5. The nature of antihæmolytic action of cholesterol and lecithin is discussed.

STUDIES ON THE ANTIHÆMOLYTIC PROPERTIES OF LECITHIN AND CHOLESTEROL.

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IN the previous report [Tsai and Lee, 1941] we have shown that the antihæmolytic function of the plasma is largely due to cholesterol. But since the antihæmolytic activity of cholesterol in the plasma as indirectly calculated can only account for about 40 per cent. of that of the whole fresh plasma, we were led to assume the existence of some other antihæmolytic substances or of some reinforcing factors acting on the antihæmolytic system of the plasma. In an attempt to elucidate this intricate problem we thought it more feasible to begin with an investigation on the various interacting factors in a simple artificial system containing cell-hæmolysin-antihæmolysin. Until more is learned regarding the antihæmolytic properties of cholesterol in such a simple medium it is not advisable to attempt to analyse the antihæmolytic mechanism of a highly complex system such as the plasma. We have incidentally observed that lecithin is hæmolytic itself, but may become antihæmolytic when mixed with saponin. Since lecithin and cholesterol are important blood constituents, their interaction in antihæmolytic behaviour should deserve our first attention.

PART I.—EXPERIMENTS WITH SAPONIN AS THE LYTIC AGENT.

Part I. of the present report deals with experiments on the antihæmolytic properties of lecithin and cholesterol against saponin. In the course of developing the technique many conditions were found to be influential, and these must be carefully observed during experimentation. It is therefore deemed necessary first to make a brief description of them.

METHODS.

Preparation of Aqueous Suspension of Cholesterol.—The oil emulsion preparation mentioned in the previous report [Tsai and Lee, 1941] does not fit our present purpose because the oil or emulsifying agent present

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in the system may influence the hæmolytic and/or antihæmolytic action, thus rendering the interpretation of data difficult. On the other hand, an aqueous suspension of pure cholesterol can be prepared which yields consistent results, and this was therefore employed in all our experiments. We found that preliminary solution in alcohol, followed by addition of water, gives a fine and stable colloidal suspension, and that subsequent evaporation of alcohol by boiling does not destroy the antihæmolytic activity of cholesterol provided the solution is not alkaline. Our procedure finally adopted was as follows: Crystalline cholesterol (E. Merck) was first dissolved in 96 per cent. redistilled alcohol (2·5 mg. per ml.). Two ml. of the solution was then removed and mixed with 20 ml. of warm water (about 60° C.). The mixture formed a colloidal suspension. The alcohol was then evaporated off by boiling the mixture over a small open flame until the volume had been reduced to 6–7 ml. After cooling, the final volume was adjusted to 10 ml. by adding distilled water. The final concentration of cholesterol was thus 50 mg./100 ml. This stock cholesterol suspension would keep unchanged for 3 or more days. Nevertheless, we always prepared a fresh sample for each experiment on the same day.

In actual experiments only 0·1 ml. of the stock solution of cholesterol was used for each 3 ml. cell-hæmolysin system so that the concentration of cholesterol in the system was only 1·67 mg./100 ml. In such dilute solution its colloidal state as well as its antihæmolytic potency remained unchanged for a considerable period of time.

Preparation of Cell Suspension.—Dog's erythrocytes were employed in all the experiments. Na-citrate was used as an anticoagulant (5 mg. for each ml. blood). The cells were washed with physiological saline. Overwashing caused hæmolysis; in most cases slight hæmolysis usually began at the fifth or sixth washing, but in some rare cases it occurred even earlier. As a rule, the more washings were made the more liability of spontaneous hæmolysis. With low-resistant cells the results are often unsatisfactory. Hence our standard method of preparing cell suspension was to wash the cells 3 times with 3 volumes of 0·9 per cent. NaCl each time. Any sample that showed a trace of hæmolysis during the last washing was discarded. If no hæmolysis took place during this washing, the cells were considered to be suitable, and were then diluted with buffered saline to a 50 per cent. suspension and used immediately.

Preparation of Saponin and Lecithin Solutions.—The hæmolytic agent used in the present experiment was saponin. The stock solution was made to 0·1 per cent. in buffered saline and used within 3 days. Just before use it was diluted to 0·02 per cent. In most experiments commercial lecithin (purchased several years ago from E. Merck) was employed both as hæmolytic and antihæmolytic agents. Fresh material prepared in our own laboratory according to the method described in

Morse's *Applied Biochemistry* was used only occasionally as a check. In all cases the material was dissolved (to make 0·05 per cent.) in buffered saline just before use.

Determination of Antihæmolytic Unit of Cholesterol and Lecithin.—Unless otherwise specified, the cell-hæmolysin-antihæmolysin system in all experiments were all of total volume of 3 ml., containing 0·1 ml. (0·05 mg.) cholesterol or lecithin solution (or 0·1 ml. water in control sample), 0·06 ml. of 50 per cent. cell suspension, and variable amounts

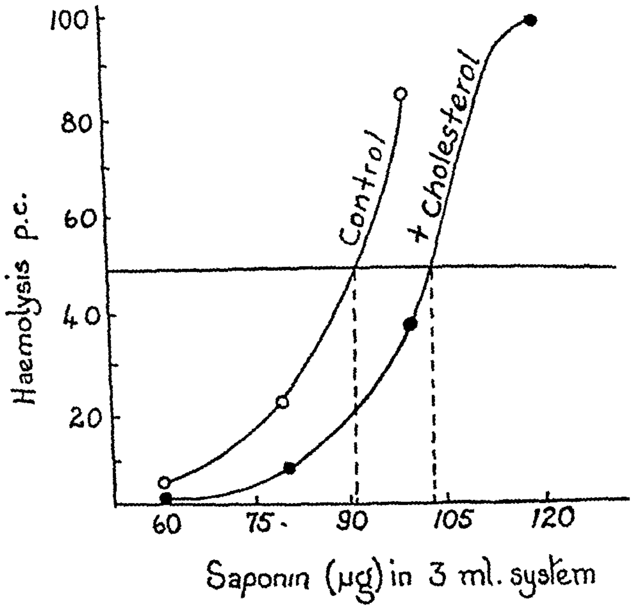

FIG. 1.—Graphs illustrating how the antihæmolytic potency of cholesterol is calculated. For explanation see the text.

of saponin and buffered saline. Saponin and saline were mixed first, followed by addition of cholesterol or lecithin. After thorough shaking, the cell suspension was then added. The mixtures were placed in a thermostatically controlled water-bath at 37° C. for 30 minutes. They were then centrifuged, and the degree of hæmolysis was determined by matching the test samples with the standards made from the same source of cell suspension. The antihæmolytic potency of 0·05 mg. of lecithin or cholesterol in the 3 ml. system was expressed in terms of micrograms (μg., or γ) of saponin inactivated by them. Fig. 1 illustrates our method of calculation. The control curve shows that to hæmolyse 50 per cent. of the cells in the 3 ml. volume requires 91·5 μg. of saponin. This quantity of saponin represents the cell resistance in μg. When 0·05 mg. of cholesterol is present in the system, the resultant curve shifts to the right side, showing 103·5 μg. saponin are now required for the production of 50 per cent. hæmolysis. The difference in the

quantity of saponin at the level of 50 per cent. hæmolysis is thus 12 μg., which is taken to represent the amount of saponin inactivated by 0·05 mg. cholesterol in 3 ml. That is the measure of the anti-hæmolytic potency of cholesterol. Similar calculation is applied to lecithin.

Cell Concentration.—The amount of saponin required for 50 per cent. hæmolysis increases with increasing concentration of the cells. However, as is shown in fig. 2, the relation of these two variables is

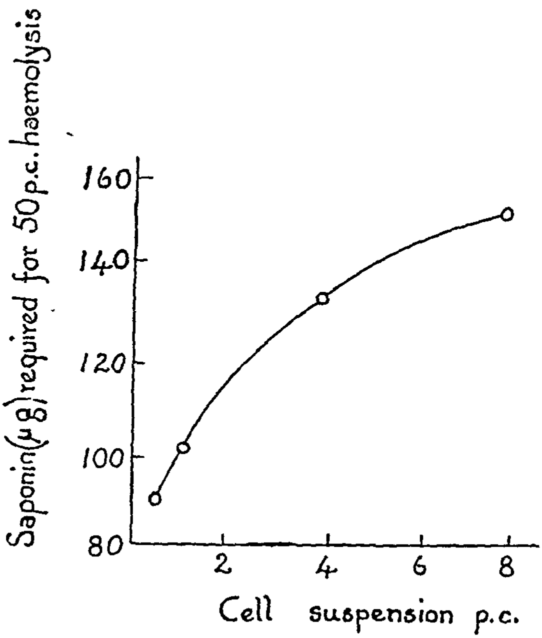

Fig. 2.—Relation of the amount of saponin required for 50 per cent. hæmolysis to cell concentration.

not linear. The milder degree of hæmolysis in higher cell concentrations may be due to the reduction of saponin concentration as a consequence of its greater dispersion or inactivation over a larger surface area of the cells. At any rate, for quantitative work cell concentration must be kept constant and uniform in different samples throughout all experiments.

Results.

Hæmolytic and Antihæmolytic Action of Lecithin.—Lecithin is an auto-oxidizable and unstable substance. Consequently the results obtained vary somewhat with different samples of material. In general it is always hæmolytic when present alone with the red blood-cells, regardless of whether the sample of material is fresh or old. However, the hæmolytic potency increases with the age of the sample. This is

illustrated by the data in Table I., where, in column 4, larger numerical values signify lower hæmolytic potency and *vice versa.* When it is mixed with saponin it may remain hæmolytic or become antihæmolytic, depending upon the freshness of the material as well as its concentration. With fresh material its predominant action is still hæmolytic, whereas with old commercial preparation it is now pre-eminently antihæmolytic. This is illustrated by the data given in the last column of Table I.

TABLE I.—THE EFFECT OF AGE ON THE ACTIVITY OF LECITHIN.

Samples.	Date of preparation.	Date of experiment.	Lecithin, μg. required for 50 per cent. hæmolysis.	Saponin inactivated, μg.
1	Commercial	Jan. 14, 1941	1920	5
2	Commercial	Mar. 6, 1941	1800	5
3	Commercial	July 4, 1941	320	0
4	July 14, 1941	July 16, 1941	750	(–)* 9

* The negative sign (–) in this and following tables signifies hæmolytic. For the derivation of hæmolytic values see Part II.

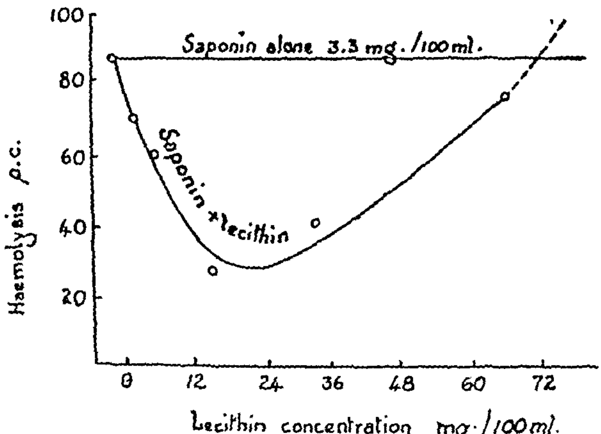

FIG. 3.—Relation of lecithin concentration in saponin solution to degree of hæmolysis. The reduction of hæmolysis at first increases with increasing concentrations up to a maximum and then gradually decreases as the lecithin content becomes higher and higher, until finally above a certain level no reduction of hæmolysis occurs. The dotted line is an extrapolation based upon the data from other experiments.

The antihæmolytic power of lecithin, when present, is rather weak, for it could never prevent hæmolysis completely, no matter what strength of it was used. When the concentration of saponin is fixed, the antihæmolytic potency of lecithin increases with its concentration

up to a certain maximum and then declines; it becomes hæmolytic again, when it is present in excess. This is shown in fig. 3. The

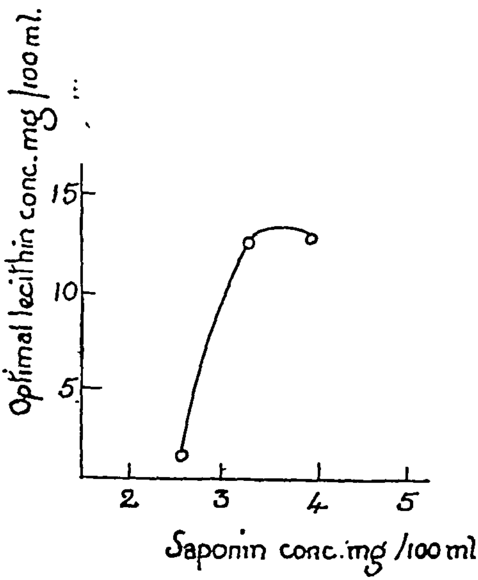

FIG. 4.—The relation of optimal lecithin concentration for antihæmolytic activity to saponin concentration.

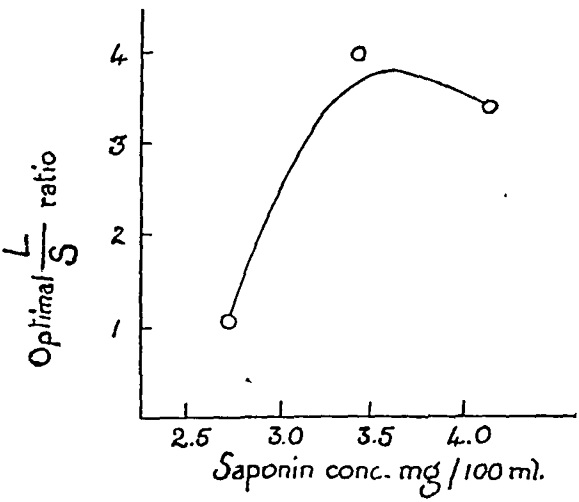

FIG. 5.—The relation of optimal $\frac{L}{S}$ ratio for antihæmolytic activity to saponin concentration.

extrapolation (dotted line) of the curve has been verified by a number of experiments and its validity cannot be doubted.

The optimal antihæmolytic power of lecithin varies not only with its own concentration, but also with that of the saponin. As illustrated by fig. 4, the optimal lecithin concentration for antihæmolytic activity increases rapidly with saponin concentration and soon reaches a maximum. With still higher saponin concentration the optimal lecithin content should be correspondingly increased. Nevertheless, the ratio of optimal concentration of these two substances is not constant; it increases up to a maximum and then declines (fig. 5).

Combined Action of Lecithin and Cholesterol against Saponin.—When both lecithin and cholesterol are present in the cell-saponin system reinforcement of antihæmolytic power is always observed regardless of the conditions of the material. It is greatest with fresh samples and becomes weaker if the material has been exposed to air for some days. A summary of one typical experiment is given in Table II. It is seen that the antihæmolytic potency of these two substances when acting together is increased approximately 3 to 10 *times*. This reinforcement does not appear to rest upon the antihæmolytic action of lecithin and may involve an independent mechanism, because it occurs even in a concentration which would have been hæmolytic but for the presence of cholesterol. This is illustrated by the data from another sample presented in Table III. In other words, the reinforcing action of lecithin on cholesterol must be due to another mechanism independently of its antihæmolytic activity.

TABLE II.—THE COMBINED ACTION OF LECITHIN AND CHOLESTEROL.

Cholesterol alone.		Lecithin alone.		Saponin inactivated by cholesterol +lecithin.		Increased inactivation of saponin, μg.
Amount in 3 ml., mg.	Saponin inactivated, μg.	Amount in 3 ml., mg.	Saponin inactivated, μg.	Calculated, μg.	Found, μg.	
0·05	12	0·05	5	17	101	84
0·05	12	0·20	33	45	287	242
0·05	12	0·50	83	95	317	222

It is of interest to note that the degree of reinforcement varies with the $\frac{\text{lecithin}}{\text{cholesterol}}$ ratio in a given range of saponin concentration. As shown in fig. 6, which is plotted from the data in Table II., the optimal ratio seems to lie round 4 or thereabout. But this optimal ratio only applies to the range of saponin concentration of 3·3–10·6 mg. per cent. With an alteration of saponin concentration the optimal $\frac{L}{C}$

TABLE III.—THE REINFORCING ACTION OF LECITHIN ON CHOLESTEROL.

Cholesterol alone.		Lecithin alone.		Saponin inactivated by cholesterol + lecithin.		Increased inactivation of saponin, μg.
Amount in 3 ml., mg.	Saponin inactivated, μg.	Amount in 3 ml., mg.	Saponin equivalent, μg.	Calculated, μg.	Found, μg.	
0·05	12	0·05	0	12	160	148
0·05	12	0·10	(−)8	4	194	190
0·05	12	0·20	(−)8	4	180	176
0·05	12	0·50	strongly hæmolytic	strongly hæmolytic	90	> 90

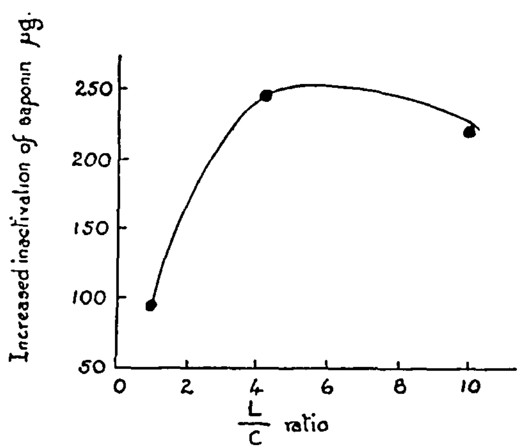

FIG. 6.—The relation of $\frac{L}{C}$ ratio to the degree of reinforcement of cholesterol action by lecithin, which is expressed in terms of an increase of the amount of saponin inactivated.

ratio also changes. The relation of these two factors has been studied in another experiment, the results of which are graphically represented in fig. 7. It is observed that the optimal ratio increases with saponin concentration, but the rate of increase is negatively accelerated.

Cholesterol is a hydrophobic colloid, while lecithin is a hydrophilic colloid; the former solution is known to be stabilized by the latter. It is quite possible that the reinforcement of antihæmolytic strength of cholesterol by lecithin may be due to this stabilizing effect. To test this hypothesis, experiments were carried out in which lecithin and cholesterol mixtures with and without saponin were allowed to incubate at 29° C. Table IV. contains the records of one of these experiments. When the three substances lecithin, cholesterol, and

saponin were incubated for 80 minutes (5), the antihæmolytic potency was higher than when incubated only for 10 minutes (3). This is not so if only lecithin and cholesterol without saponin (4) are incubated for similar length of time, indicating that reinforcement of cholesterol

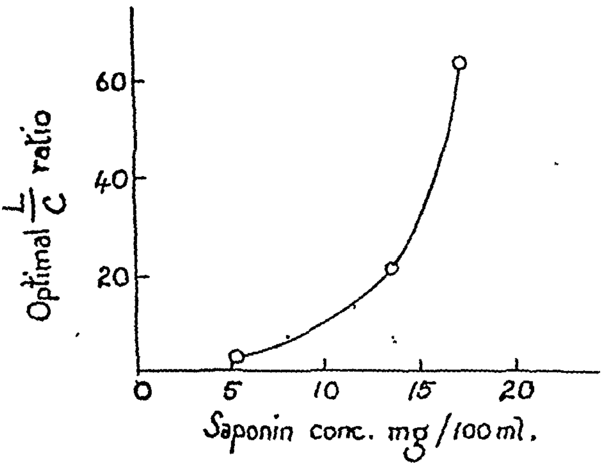

FIG. 7.—The relation of optimal $\frac{L}{C}$ ratio for antihæmolytic reinforcement to the concentration of saponin.

TABLE IV.—REINFORCEMENT OF CHOLESTEROL BY LECITHIN.

Substances.	Time of incubation, minutes.	Antihæmolytic potency in saponin inactivated, μg.
(1) Saponin alone	..	0
(2) Saponin + cholesterol	10	12
(3) Lecithin + cholesterol + saponin	10	125
(4) Lecithin + cholesterol	70, then saponin added, further 10	102
(5) Lecithin + cholesterol + saponin	80	165

action by lecithin takes place only in the presence of saponin. It is therefore very unlikely that the stabilizing action of lecithin can be the contributory factor in the reinforcement of cholesterol. Furthermore, as shown in the next section, lecithin is hæmolytic in the presence of cholesterol alone.

The Interaction of Lecithin and Cholesterol in a Lysin-free System.—In the preceding sections we have demonstrated that lecithin itself is hæmolytic and may remain hæmolytic or become antihæmolytic in the presence of saponin. It remains interesting to see whether the antihæmolytic effect of lecithin still occurs in the absence of saponin.

For the settlement of this point experiments were carried out in which saponin was omitted in the hæmolysin-antihæmolysin system—that is, lecithin was used as the hæmolytic agent and cholesterol as the antihæmolytic. In these cases lecithin remains hæmolytic and may be inhibited by cholesterol.

Discussion.

Levin [1935] has shown that pure fresh lecithin is antihæmolytic, whereas old commercial preparations are hæmolytic, especially in dilute solutions. Although the purity of our materials was not ascertained, our results are consistent, and differ from those of Levin in showing that fresh material is predominantly hæmolytic, whereas old commercial preparation may be either hæmolytic or antihæmolytic, depending upon its age and concentration. As lecithin is an auto-oxidizable and unstable substance, variation in its hæmolytic and antihæmolytic behaviour is to be anticipated. Nevertheless, we shall seek an early opportunity to repeat the above experiments with the crystalline substance.

Ponder [1934] has shown that when two lysins are present in the system the resultant action toward the cells may be mutual inhibition or acceleration. Thus, for instance, saponin + digitonin or + taurocholate are inhibitory and saponin + oleate and taurocholate + glycocholate are acceleratory. He postulated that the resultant action is due to the interaction of the two substances with cell constituents. The antihæmolytic action of lecithin against saponin hæmolysis reported in the present communication may be considered similarly as an outcome of interaction of these two lysins. As we shall describe in the next section, the behaviour of lecithin toward other lysins is not invariably antihæmolytic.

The most important fact demonstrated in this series of experiments is the reinforcement of the antihæmolytic activity of cholesterol by lecithin. In our previous paper we have emphasized the importance of cholesterol in the antihæmolytic action of the plasma and realized the complexity of the mechanism. We have now obtained evidence which leads to the suggestion that lecithin may also play an essential rôle in the protection of red blood-cells against the attack of lysins. One must, however, be aware that lecithin cannot prevent saponin hæmolysis completely without cholesterol. This indicates that it must act as an adjuvant of cholesterol in order to protect the cells with absolute certainty. As to how it behaves in the living body we do not know. Until we learn more about the conditions for the manifestation of reinforcement we must content ourselves without attempting further postulation and application.

SUMMARY.

1. Methods of preparing a simple artificial cell-hæmolysin-antihæmolysin system are described, and various influential factors reported.

2. Lecithin when allowed to act alone on the washed dog's red blood-cells is hæmolytic, but may remain hæmolytic or become antihæmolytic when saponin is present in the system, depending upon the freshness of the material and its concentration. With fresh material the reaction is predominantly hæmolytic, whereas with old commercial preparation it is eminently antihæmolytic, especially in dilute solutions.

3. The antihæmolytic activity of cholesterol is reinforced by lecithin (3–10 times). This reinforcement does not depend upon the antihæmolytic action of lecithin, and appears to be controlled by another mechanism. It is not due to the stabilizing action of lecithin on cholesterol solution.

4. The extent of reinforcement depends upon the ratio of $\frac{\text{lecithin}}{\text{cholesterol}}$ in a given range of saponin concentration. The ratio increases with an increase of saponin concentration up to a certain maximum and then declines.

5. Without saponin, lecithin remains hæmolytic in the cell-cholesterol system.

6. The antihæmolytic behaviour of lecithin toward saponin, and the reinforcing action on cholesterol, must be attributed to its interaction with saponin, because without the latter these actions cannot occur.

PART II.—EXPERIMENTS WITH OTHER LYTIC AGENTS.

In this part we desire to communicate some similar experiments on the antihæmolytic action of lecithin and cholesterol against other lytic agents. Lecithin used in this series of experiments was an old commercial preparation freshly removed from the stock bottle. The hæmolysins we have studied were digitonin, crude bile acid, sodium taurocholate, sodium glycocholate, oleic acid, and natural hæmolysin. The procedure of all the experiments was exactly the same as that described in Part I. Dog's citrated plasma or serum was employed as a natural hæmolysin against rabbit's washed blood-cells. In all other experiments washed dog cells were used. The antihæmolytic activity of a definite quantity of lecithin and cholesterol (unless otherwise specified it is usually 0·05 mg.) was expressed in terms of micrograms (μg.) of the lysin inactivated in the 3 ml. system. When lecithin was hæmolytic in the presence of another lytic agent its potency was expressed also in microgram equivalents of that particular lysin, but to distinguish it from that inactivated a negative sign (–) was added

in front of the numerical value. For example, in one experiment it required 50 μg. of oleic acid in the 3 ml. system to produce 50 per cent. hæmolysis of the cells when oleic acid was present alone. When this lysin was mixed with lecithin it required only 25 μg. oleic acid to cause the same percentage of hæmolysis. Hence the hæmolytic strength of lecithin in this particular case was equivalent to 25 μg. oleic acid. For convenience we wrote it as (–) 25.

RESULTS.

Oleic Acid and Bile Salts.—Table V. gives the results of three typical experiments dealing with oleic acid, taurocholate, and glycocholate respectively. Lecithin appears to behave similarly toward these three

TABLE V.—THE EFFECT OF CHOLESTEROL AND LECITHIN ON OLEIC ACID, TAUROCHOLATE, AND GLYCOCHOLATE HÆMOLYSIS.

	Lysin equivalent or inactivated.	
	Found, μg.	Calculated, μg.
(1) Oleic alone	0	..
(2) Oleic acid + lecithin (0·1 mg.)	(–) 25	..
(3) Oleic acid + cholesterol	32	..
(4) Oleic acid + lecithin (0·1 mg.) + cholesterol	25	7
(1) Na-taurocholate alone	0	..
(2) Na-taurocholate + lecithin	(–) 160	..
(3) Na-taurocholate + cholesterol	440	..
(4) Na-taurocholate + lecithin + cholesterol	370	280
(1) Na-glycocholate alone	0	..
(2) Na-glycocholate + lecithin	(–) 245	..
(3) Na-glycocholate + cholesterol	400	..
(4) Na-glycocholate + lecithin + cholesterol	180	155

lysins in that it remains hæmolytic in all of them, though its hæmolytic power in each lysin system varies to some extent with different samples. With the presence of cholesterol the reinforcing effect of lecithin was observed in most cases. Thus, for instance, in the experiment presented in the first section of Table V. lecithin (0·1 mg.) has exercised a hæmolytic action equivalent to (–) 25 μg. oleic acid and cholesterol an antihæmolytic action equivalent to an inactivation of 32 μg. oleic acid. If the hæmolytic strength of lecithin remains unchanged in the presence of cholesterol, the sum of lecithin and cholesterol activities should be an inactivation of 7 μg. oleic acid. Since the observed value is 25 instead of 7, we assume that the hæmolytic action of lecithin is

reduced by cholesterol or the antihæmolytic power of the latter is reinforced by the former. Both these explanations are equally valid, although for the sake of consistency we prefer to adopt the latter. The same calculation and interpretation also apply to taurocholate, glycocholate, and other hæmolysins.

Crude Bile Acid Preparation.—This was prepared according to the method described in Mathews' *Physiological Chemistry* (1930). Its purity was not ascertained. But as its action differs from taurocholate and glycocholate, we thought it worth while to report our observation.

As shown in Table VI., crude bile acid preparation is hæmolytic. The behaviour of lecithin and lecithin + cholesterol toward it is similar

TABLE VI.—THE EFFECT OF CHOLESTEROL AND LECITHIN ON HÆMOLYSIS CAUSED BY CRUDE BILE ACID AND MIXTURE OF TAUROCHOLATE AND GLYCOCHOLATE.

	Lysin equivalent or inactivated.	
	Found, μg.	Calculated, μg.
(1) Crude bile acid alone	0	..
(2) Crude bile acid + lecithin	40	..
(3) Crude bile acid + cholesterol	20	..
(4) Crude bile acid + lecithin + cholesterol	75	60
(1) Taurocholate + glycocholate (1 : 3)	0	..
(2) Taurocholate + glycocholate + lecithin	(−) 300	..
(3) Taurocholate + glycocholate + cholesterol	500	..
(4) Taurocholate + glycocholate + lecithin + cholesterol	140	200

to that toward saponin, *i.e.* lecithin is antihæmolytic to crude bile acid and its antihæmolytic power in this particular case is greater than that of cholesterol. Furthermore, when lecithin and cholesterol are present in this lytic system they reinforce each other in antihæmolytic action.

Since lecithin is hæmolytic both in taurocholate and glycocholate, its antihæmolytic action toward crude bile acid must be due either to the impurity of the preparation or the interaction of taurocholate and glycocholate. To test the latter possibility several experiments were performed in which taurocholate and glycocholate in a proportion of 1 : 3 were mixed with lecithin, cholesterol, and lecithin + cholesterol respectively, and their resultant actions were determined as usual. The results from one of such experiments are incorporated in Table VI. It is obvious that lecithin is hæmolytic in taurocholate and glycocholate mixture, and it exerts practically no reinforcing action on cholesterol in such a system. Therefore its antihæmolytic behaviour toward crude

bile acid cannot be ascribed to the interaction of taurocholate and glycocholate, and must be due to some other unknown factor.

Digitonin.—Digitonin is known to be a strong hæmolytic agent. The behaviour of lecithin toward this lysin closely resembles that toward saponin; it is markedly antihæmolytic in digitonin and exercises a strong reinforcing action on cholesterol activity similar to saponin. This is shown in Table VII.

TABLE VII.—THE EFFECT OF CHOLESTEROL AND LECITHIN ON DIGITONIN HÆMOLYSIS.

	Digitonin inactivated.	
	Found, μg.	Calculated, μg.
Digitonin alone	0	..
Digitonin + cholesterol	25	..
Digitonin + lecithin	26	..
Digitonin + cholesterol + lecithin	200	51

Natural Hæmolysin.—It has long been known since the work of Bordet that the serum of one animal may hæmolyse the washed red blood-cells of another animal of a different species. We have found that this type of hæmolysis can also be prevented by the plasma of the latter animal, by lecithin, and by cholesterol. The resistance of rabbit's cells against dog's plasma or serum and the hæmolytic power of the latter vary with different individuals as well as many other conditions. Speaking generally, hæmolysis caused by 1 ml. of dog's citrated plasma can be completely prevented by 2 mg. cholesterol or 1·5–2·0 ml. of rabbit plasma. Lecithin is antihæmolytic to dog's plasma. The inactivation of the hæmolytic serum by lecithin, cholesterol, and these two substances together is shown in Table VIII., where the inactivation

TABLE VIII.—THE EFFECT OF CHOLESTEROL AND LECITHIN ON HÆMOLYTIC SERUM.

	*Serum inactivated.**	
	Found, μl.	Calculated, μl.
Serum alone	0	..
Serum + cholesterol (0·2 mg.)	40	..
Serum + lecithin (0·2 mg.)	40	..
Serum + cholesterol + lecithin	60	80

* The numerical values below are expressed in terms of microlitres of citrated plasma.

is expressed in terms of microlitres (μl. or c.mm.) of serum. Here we see no reinforcement of cholesterol by lecithin.

The hæmolytic activity of the plasma is known to be due to the interaction of amboceptor and complement. Which of these elements is inactivated by lecithin and which by cholesterol? We shall report our experiments on this question in a separate communication.

Discussion.

Cholesterol is antihæmolytic to all chemical lysins studied. The significance of this finding should be emphasized. One might at first think that cholesterol acts on the cell membrane so as to protect it or prevent it from the invasion and attack of various lytic agents. But our results do not substantiate this hypothesis, for cholesterol is found to react with the lysins before the addition of the cells. In the second place, the degree of inactivation of different lysins varies. This is shown in Table IX., where the numerical values were derived from different experiments of a similar nature. Although they are not strictly comparable, some rough idea may be gained from these computed data. If cholesterol acts on the cells alone, one should not expect such an extreme variation in the degree of inactivation of different lysins. We therefore believe that in many cases an interaction between cholesterol and the lytic agents must have taken place independently of the cells. If this proves to be true, then the possibility is chloresterol combines directly with the lysins. It seems unlikely it can combine with all of these chemically widely different substances.

Table IX.—Inactivation of Various Lysins by Cholesterol at Room Temperature.

	Amount required for 50 per cent. hæmolysis in 3 ml., μg.	Amount inactivated in 3 ml. by 0·05 mg. cholesterol, μg.	Percentages inactivated.
Digitonin	14	25	178
Lecithin	250	220	88
Oleic acid	45	27	60
Na-glycocholate	770	374	49
Na-taurocholate	880	380	43
Natural hæmolysin*	260	110	42
Saponin	90	12	13
Crude bile acid	220	20	9

* The numerical values in this row are expressed in terms of microlitres of citrated plasma.

While its antihæmolytic process against different lysins may differ in each case or in each group of substances, some may be chemical and some physical. Our experiments to be described in the following paper appear to verify this assumption.

Lecithin is antihæmolytic toward saponin, digitonin, crude bile acid, and natural hæmolysin, but remains hæmolytic in oleic acid, taurocholate, and glycocholate systems. In the first three substances of the former group the antihæmolytic action of cholesterol is reinforced by lecithin; the antihæmolytic activity and reinforcing action are most striking in saponin and digitonin. In the latter group the reinforcement is comparatively feeble and is manifested only by the reduction of the hæmolytic activity of lecithin. In other words, when both cholesterol and lecithin are present in a lytic system, the reinforcing effect occurs regardless of whether lecithin itself is hæmolytic or antihæmolytic toward that particular lysin originally. This is consistent with our statement that the reinforcement of cholesterol action by lecithin is independent of the antihæmolytic mechanism of the latter.

The antihæmolytic action of lecithin toward a number of lysins and its reinforcing effect on cholesterol have marked its importance in the antihæmolytic behaviour of the plasma. The antagonistic action of lecithin toward hæmolytic plasma suggests a likelihood of a similar function under normal condition in the plasma. It explains at least in part why we were unable in our previous investigation to account for the antihæmolytic potency of fresh plasma by the activity of cholesterol alone. We are certain that, besides lecithin and cholesterol, there are other substances and factors that share the antihæmolytic activity of the plasma, and hope that by similar methods of tackling the problem we may be able to explore the field more thoroughly and throw more light on the intricate mechanism under consideration.

Summary.

1. Lecithin is hæmolytic toward oleic acid, taurocholate, and glycocholate, but antihæmolytic to digitonin, crude bile acid preparation, and natural hæmolysin (dog's citrated plasma or serum against rabbit's corpuscles).

2. The antihæmolytic behaviour of lecithin toward crude bile acid is not due to the interaction between taurocholate and glycocholate.

3. The antihæmolytic activity of cholesterol is reinforced by lecithin in all lysin systems except natural hæmolysin, regardless of whether lecithin is inhibitory or acceleratory toward them in the absence of cholesterol. The reinforcement is most pronounced in saponin and digitonin. It is also very evident in crude bile acid and oleic acid. But it is rather feeble in Na-taurocholate and Na-glycocholate.

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THE CHEMICAL EXCITABILITY OF THE ISOLATED RABBIT SMALL INTENSTINE.

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The theory of the gradient of excitability of the intestine advocated by Alvarez [1928] to explain the aboral direction of peristalsis was mainly based on the differences in rate of the rhythmic contraction of the intestine from different levels both *in vivo* and *in vitro*, together with some evidence indicating differences in metabolic rate. This has been further corroborated by the finding that in the intact intestine of the rabbit a gradient of electric excitability can actually be demonstrated, the upper end being more excitable than the lower end [Alvarez and Hosoi, 1929 *a*].

In view of the great sensitiveness of smooth muscles to chemical stimuli, the question naturally arises whether the excitability to chemical agents of the smooth muscles of intestine is also consistent with the gradient theory. Although much work has been reported on the effects of various drugs on intestinal motility, little attention has been paid to this comparative aspect of the problem. Since the advent of the theory of chemical transmission of nerve impulses, the facts of the chemical excitability of tissues have acquired greater physiological significance, for the two active substances acetylcholine (AC) and adrenaline (Ad) are no longer considered respectively as a foreign substance or as one produced in quantity only during emergency, but are thought to be constantly produced and destroyed in every innervated tissue, and by their local action to be the ultimate regulators of cellular activity. The sensitivity of tissues to these stimuli would in a sense determine the power of nervous control over them.

The present investigation was carried out with a view to studying the relative excitability to chemical stimuli of the longitudinal smooth muscle of the isolated rabbit small intestine, taken from different levels of the intestine, with special reference to AC and Ad.

Experimental Method.

Adult rabbits of both sexes weighing from 1 to 2 kg. were used. Half an hour before the experiment they were usually given a dose of

chloral hydrate (3 to 5 c.c. of 25 per cent. solution) intramuscularly. The abdomen was opened, sometimes with the aid of a little ether, warm saline was poured into the cavity and samples of intestinal segments were then taken between ligatures while vigorous peristalsis was going on. The segments were temporarily kept in cold Tyrode solution. When the necessary arrangements were ready they were transferred to a bath containing warm, aerated Tyrode or Locke solution with 0·1 per cent. glucose and contractions recorded with light levers. One, two, or three segments from different levels of the gut were studied at a time. The segments were referred to as duodenum (D), jejunum (J), or ileum (I) respectively as they were sampled from the upper, middle, and lower levels of the small intestine. Drug solutions were introduced with a micropipette directly into the bath and quickly dispersed by an air current. The AC solution was prepared from acetylcholine chloride (E. Merck) and Ad solution diluted from ampoules of 1/1000 adrenaline hydrochloride (International). The bath fluid was usually changed after each observation and enough time was allowed for complete recovery before the next observation.

Results.

1. *Some Preliminary Remarks on the Motility of the Isolated Intestinal Segments.*

The general behaviour of the Magnus preparation is too familiar to require description in detail. Herewith we only record some of the more pertinent points.

(a) *Rhythmicity.*—As is well known, raising the temperature of the bath increases the rhythm of contraction of the isolated intestine. This property is shared about equally by all the segments. However, the relation between rate and temperature is not linear (fig. 1). The average values indicate that an increase in temperature of 10° C. approximately doubled the rate of contraction. The range of temperature compatible with regular co-ordinated rhythm lay about between 22° and 45° C. On approaching the upper range the rhythmic contractions gradually gave way to small inco-ordinated twitchings, but might recover again if enough time be allowed for adaptation.

Another condition affecting rhythm is the hydrogen ion concentration. The pH of the Tyrode solution was varied by changing its content of bicarbonate and acid phosphate. It was observed that a fall of pH caused slowing of rhythm. This effect was most marked at the lower levels of the intestine. In one extreme case when the pH was changed from 8·5 to 6·1 the contraction rate of the ileum dropped from 15 to 2 per minute, and that of jejunum from 16 to 10, while that of duodenum remained at 21 without change.

In all subsequent experiments the pH of the bath solution was

kept around 8·5 and the temperature about 38° C. Aside from a gradual spontaneous slowing-down of the duodenum rhythm during the initial period of the experiment the rhythm usually suffered no other change. Neither was it affected by drug action in the concentrations used in our experiments.

(b) *Tone and Amplitude.*—The tone of the intestinal segments depended to a great extent on the nature of the solution in the bath.

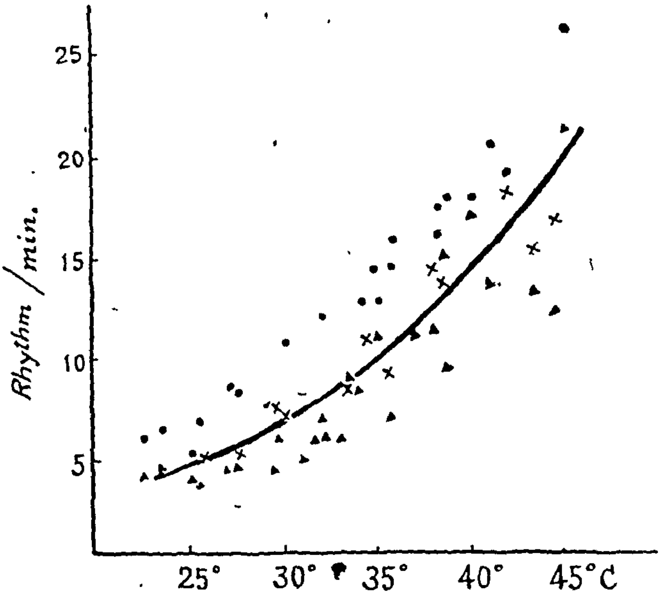

FIG. 1.—The relation between temperature of the bath and rhythm of contraction of the isolated rabbit intestine. (●) duodenum; (×) jejunum; (▲) ileum. Results from four experiments.

In Locke solution contractility was good, but often with fluctuations of tone, especially in the duodenum and jejunum. The amplitude of contraction was also irregular. In Tyrode solution the amplitude became smaller but more uniform, and spontaneous fluctuation of tone was reduced to minimum. For the purpose of comparing drug actions we thought it better to use Tyrode solution, prepared from glass distilled water and pure chemicals. In such solution it was noted that, with apparently equal lengths of muscle fibre, the amplitude of contraction was usually greatest in the ileum. The jejunum contraction, on the other hand, was weak and sometimes the preparation had to be discarded.

2. *The Sensitivity of the Different Segments to AC and Ad.*

The action of AC and Ad on smooth muscle is well known. However, if one tests their actions on the spontaneously contracting muscle

from various levels of the gut and uses the smallest concentration which produces a perceptible effect on the tone and amplitude of their contraction as a criterion of sensitivity, one discovers that the sensitivity is not uniform *throughout the whole small intestine:* the duodenal muscle is more sensitive to AC, while the ileal muscle is more sensitive to *Ad.* This is shown in Table I., which summarises the results of preliminary observations. In these experiments the segments were removed from the anæsthetised animals and tested in the Magnus bath one after another, *sometimes with the duodenum first and sometimes with the ileum first.*

TABLE I.—MINIMAL EFFECTIVE CONCENTRATION OF AC AND AD ON ISOLATED RABBIT INTESTINE.

(Micrograms per litre.)

Exp. No.	AC.			Ad.		
	D.	J.	I.	D.	J.	I.
1	0·5	..	3·0	12·0	..	12·0
2	2·5	..	2·5	5·0	..	5·0
3	1·8	3·1	3·6	7·5	5·0	5·0
4	0·3	0·6	0·6	5·0	2·5	2·5
5	0·6	1·8	3·0	10·0	7·5	5·0
6	1·2	1·2	1·8	10·0	7·5	5·0
7	2·5	..	2·5	..	5·0	..
8	0·01	..	0·05	10·0	..	5·0

Thus a majority of the results show a topographical difference in sensitivity. *If this difference can be called the gradient of excitability,* the gradient is from above downwards for AC and from below upwards for Ad. The few negative results do not invalidate this main conclusion since there are apparently various conditions affecting the exhibition of the phenomenon which have not been under control in these *experiments.*

Among the positive results there existed indeed a great deal of individual difference in the sensitivity to AC. The difference was exhibited even in the same intestine under various conditions. In one experiment we observed a decline of sensitivity to AC in the second lot of intestinal segments sampled from the anæsthetised animal half an hour after the removal of the first lot. The sensitivity to Ad in this case, however, remained unchanged. The only noticeable difference between these two lots was that when the first lot of samples were taken active peristalsis was seen, whereas at the time of removing the second lot the animal was not in good condition and there was no

peristaltic movement. In subsequent experiments this source of error was eliminated altogether by registering the motility of various segments simultaneously in the same bath. During the addition of drug solutions care was taken that they should reach the different pieces of intestine at the same moment and in equal concentration. Fig. 2 is a typical experiment in which the duodenum is seen to be more sensitive to AC and least sensitive to Ad.

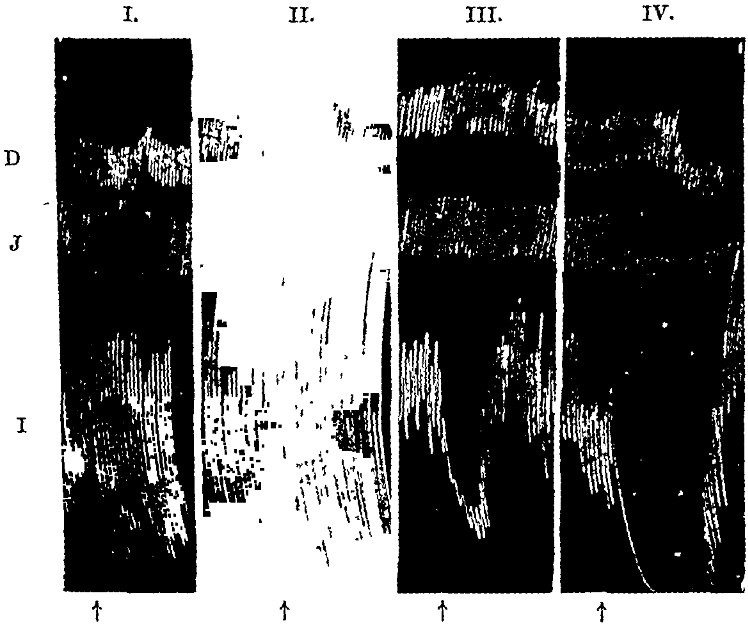

FIG. 2.—Contractions of duodenum, jejunum, and ileum. Bath capacity, 100 c.c. I. 0·04 c.c. of AC, 1:400,000. II. 0·06 c.c. of same. III. 0·4 c.c. of Ad, 1:100,000. IV. 5 c.c. of same.

3. *Comparison of the Action of AC and Ad with other Drugs.*

We were next concerned with the comparison of AC action with those of other stimulants and of Ad action with those of other depressants. The first class consisted of choline, pilocarpine, eserine, histamine and barium, and the second class of atropine, magnesium, and potassium. We found that the excitability of intestinal muscle to these agents is somewhat different from those described above for AC and Ad. As the difference is only quantitative rather than being qualitative, three criteria may be used for comparison: (*a*) the criterion described in the last section, viz. the threshold concentration; (*b*) the relative change of amplitude or tone in response to the drug action; and (*c*) the period of persistence of the effect. The last two measure the intensity of the response.

(a) *Threshold Concentration.*—The minimal effective concentration of barium chloride is practically the same for the upper and lower segments. This is well illustrated in fig. 3, in which the dosage of stimulants has been so adjusted as to exert a comparable elevation of tone in the duodenum (*i.e.* 1 with 3 and 2 with 4). It will be seen that while the ileal response to AC in 1 is far from being significant, its response to barium in 3 is quite marked. This would mean that, unlike AC, the whole length of small intestine is equally sensitive to barium.

(b) *Amplitude and Tone.*—The response of the intestinal muscle to a stimulus is manifested in various ways; it may be an increase of amplitude, or a rise of tone, or a mixture of the two. Fig. 3 shows the

FIG. 3.—Contractions of duodenum (upper curve) and ileum (lower curve). Bath capacity 100 c.c. At arrow 1, 0·08 c.c of AC, 1 : 400,000. At arrow 2, 0·1 c.c. of same. At arrow 3, 0·005 c.c. of 5 per cent. $BaCl_2$. At arrow 4, 0·008 c.c. of same.

tone response of both the duodenum and ileum to AC and barium. While the increase of tone of the ileum is smaller than that of the duodenum in response to AC (1 and 2), it is of the same order of magnitude on barium stimulation (3 and 4). Fig. 4 shows a preparation in which there was hardly any change in tone, but the amplitude of contraction of the duodenum increased enormously on giving a moderate dose of stimulant. The increase of amplitude of the ileum was, however, insignificant. This latter phenomenon may be due to the fact that the contraction of this segment had already approached a maximum before the addition of the drug so that a further increase was difficult. However, it can still be seen that the relative increase of amplitude of the ileum was smaller after AC (2 and 3) than after barium (1).

(c) *The Period of Persistence of the Effect.*—When the intestine is stimulated with a small dose of AC, the effect passes away quickly. With a bigger dose, the contraction of the upper segments undergoes augmentation for a considerable length of time; the effect on the ileum

however, is quite transitory. This, however, is not the case with barium as a stimulant. Fig. 4 compares the period on persistence of the stimulant action of AC (2 and 3) and barium (1) on the ileum. The stimulating action of barium on the ileum is quite as long as on the duodenum.

A consideration of the evidence adduced above warrants the conclusion that the response of intestinal muscle to barium differs from that to AC in showing absence of a gradient of excitability.

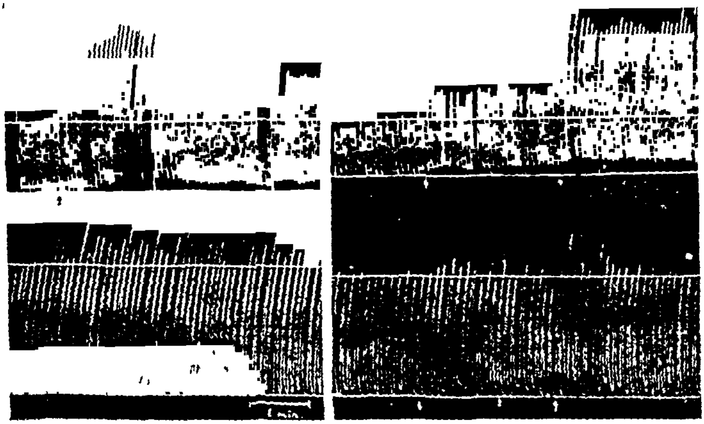

FIG. 4.—Contractions of duodenum (upper curve) and ileum (lower curve). Bath capacity 100 c.c. At arrow 1, 0·002 c.c. of 5 per cent. $BaCl_2$. At arrow 2, 0·065 c.c. of AC, 1 : 400,000. At arrow 3, 0·13 c.c. of same.

We have alto tested a few of the other common stimulants. Pilocarpine hydrochloride in a concentration of 0·05 mg./l. and upward caused a strong and long-lasting stimulation of both the duodenum and ileum. Physostigmine salicylate (0·1 mg./l.) raised the tone of both. Histamine dihydrochloride (about 5 mg./l.) also stimulated both upper and lower segments of the intestinal muscle. The effect of choline chloride (0·1 mg./l.) was slow and long-lasting and of approximately the same magnitude in the duodenum as in the ileum. It is remarkable, therefore, that among the drugs tested only AC shows some topographical difference in action on the intestine, whereas the other parasympathomimetic drugs whose actions are so akin to AC failed to exhibit a similar phenomenon. Similarly the actions of intestinal depressants other than Ad so far studied—potassium chloride, magnesium chloride, and atropine sulphate—all acted about equally on all segments of the intestine (fig. 5). The action of atropine is unique in that with a single large dose inhibition occurred, but if the dosage was gradually increased from a minimum a *much higher concentration*

could be tolerated without diminishing the tone and amplitude of contraction. After atropine, the threshold to AC stimulation was enormously raised and the gradient of excitability to this drug disappeared.

FIG. 5.—Contractions of duodenum, jejunum, and ileum. Bath capacity 100 c.c. At 1, 2 c.c. of 2·4 per cent. KCl. At 2, 0·5 c.c. of Ad, 1 : 100,000.

4. *Factors Affecting the Gradient of Excitability to AC and Ad.*

We must admit that the gradient of chemical excitability was not a uniform feature in our experiments (see Table I.). In some cases the sensitivity of the different intestinal segments to AC and Ad was more or less similar and in rare cases the lower level was more excitable to AC than the upper level. This divergence from the gradient may in some way be related to the disturbance of functional balance of the intestine. Alvarez and Hosoi [1929 *b*] reported that the gradient of electric excitability of the intact intestinal muscle was unstable and could be reversed by previously irritating the distal end of the ileum. In a few experiments we have tried to reproduce this effect by injecting

oil of turpentine into the sacculus rotundus. After two to five days the animal was sacrificed and segments of intestine were removed for observation as usual. In some instances we found that ileum was not more sensitive to Ad than duodenum, but in no case was the phenomenon of reversal observed.

Some incidental observations lead us to believe that the method of obtaining the intestine might have played a rôle in determining its

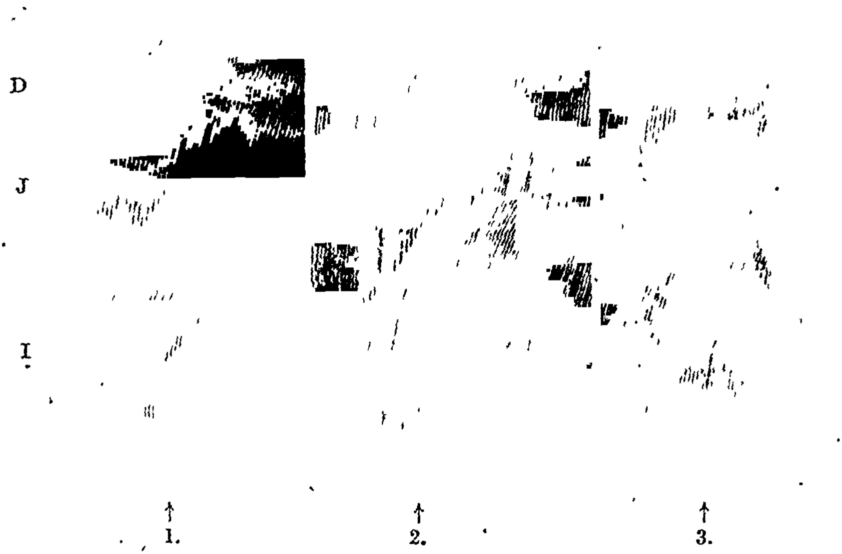

FIG. 6.—Contractions of the previously asphyxiated duodenum, jejunum, and ileum. 1, 0·08 c.c. of AC, 1 : 400,000; 2, 0·0025 c.c. of 5 per cent. $BaCl_2$; 3, 2 c.c. of Ad. 1 : 100,000.

subsequent sensitivity. If one killed the animal according to the conventional method by a blow on the head with a hammer and removed the intestines from the carcase, the tissue was found under profound inhibition and asphyxia. Such segments, although able to recover motility in the warm aerated solution subsequently, were mostly abnormal in behaviour. The material used in the experiment shown in fig. 6 was obtained in this way. It will be seen that not only the gradient of chemical excitability has been upset, but the difference in the rhythm of the upper and lower segments also vanished. A closer examination of the record reveals that the rhythm of the ileum was actually higher than that of the duodenum and so was the intensity of reaction to AC, suggesting a positive correlation between rhythmicity and chemical excitability. It has been pointed out above that the sensitivity of intestine muscle to AC was diminished while the animal was under prolonged anæsthesia and in a bad condition. Taking

the evidence together it becomes quite possible that such factors as asphyxia and shock are able to damage the characteristics of chemical excitability of the muscle. A longer period of asphyxia, as has been pointed out by Ascanio and Alvarez [1929], is detrimental even to the subsequent recovery of motility.

Discussion.

The present investigation offers evidence to show that there is a gradient of excitability of the intestinal smooth muscle to AC and Ad, and that the action of other stimulants and depressants is about uniform in intensity on this muscle. This finding of a gradient of chemical excitability to particular drugs is not entirely a novelty. Alvarez [*loc. cit.*] has already observed that the period of persistence of the inhibitory action of adrenaline was longer in the lower part than in the upper part of the rabbit isolated intestine. But he explained it as indicating a higher irritability of the upper segments which were more ready to recover from the inhibition. This, however, can hardly explain the inhibitory action of potassium. Bernheim [1934] reported a higher sensitivity of rat's ileum to adrenaline, but was not able to confirm it in the rabbit's intestine. No one, however, seems to have taken notice of the gradient in sensitivity to acetylcholine. This is probably due to the fact that the AC gradient most easily deteriorates in the process of preparation of the intestine, a fact already alluded to. Parallel with the rapid loss of excitability to AC we have recorded the initial decline of rhythm of the isolated duodenum. When the order of rhythm was reversed, the excitability to AC was reversed too (fig. 6). The last two facts suggest some relationship between the relative rate of rhythmicity and excitability to AC.

One may proceed to offer some explanation for this difference of excitability to AC and Ad in the Magnus bath. Three possibilities need be considered. (1) The rate of inward diffusion of drugs into the muscle tissue may be different. There is little data pertaining to the permeability of the intestinal serosa to AC and Ad. However, this is probably not an important factor since it is not known that a membrane more permeable to AC is less permeable to Ad. (2) The rate of destruction of drugs in the tissues may be different. This is suggested by the long-lasting effect of AC on duodenum muscle in contrast to its short-lived action on ileum (fig. 4). If it could be demonstrated that the duodenum muscle contains more amine exidase, or other enzyme system capable of destroying adrenaline, and that the ileum muscle contains more choline esterase, or other *mechanism capable of hydrolysing or* oxidizing AC, the phenomenon is easily explained. Unfortunately there is no available information on this point. Bernheim [1934] ventured to infer that the intestinal muscle is devoid of choline esterase.

This seems unlikely in view of the abundance of choline content of the intestine [LeHeux, 1919, quoted by Henderson and Roepke, 1937]. However, the question can easily be settled by actual determination. (3) The reactivity of muscle cells of the different segments may differ. It is conceivable that the smooth muscle fibre of the upper end of the small intestine is slightly different in nature from that of the lower end. The differences in their rates of contraction, and in metabolism and electric excitability being known, a difference in chemical excitability is not improbable. The positive correlation between rhythm and excitability to AC is an evidence for this hypothesis.

It is generally taught that autonomic drugs such as adrenaline, pilocarpine, and atropine act on the "receptive mechanism," while direct stimulants such as barium act on the "responsive mechanism." Acetylcholine is believed to belong to the former class. The present results, however, do not seem to show any difference in the general reactivity of the two mechanisms between upper and lower part of the intestine, since their response to most autonomic drugs as well as to direct stimulants and depresents are about the same, but, on the other hand, they do raise a sharp distinction between chemicals capable of being produced in the body and those of foreign origin. This is probably more of physiological than of pharmacological significance. So far as the exact mechanism of excitation and inhibition is unknown it can only be conjectured that each chemically distinct drug has its own way of working into the mechanism of cellular function.

From the standpoint of animal economy, an opposite gradient of sensitivity to AC and Ad would imply a relative easiness of the upper intestine to undergo excitation and of the lower intestine to undergo inhibition. Evidence is at hand that stimulation of sympathetics to the small intestine is accompanied by the liberation of Ad or Ad-like substance [Finkleman, 1930], and that stimulation of the vagus is accompanied by the liberation of AC [Bunting, Meek, and Masake, 1935]. If autonomic impulses take effect only through these chemical mediators, the sympathetic control would be more effective in the lower part of the small intestine and the parasympathetic more in the upper part, hence a better chance for the duodenum to empty its contents to the ileum at any phase of autonomic activity. This implies of course that the excitability of the muscle tissue around the nerve endings also shows the phenomenon of gradient as the muscle fibre in general, which is highly probable. However, as no data is available regarding the relative number of autonomic nerve endings in the upper and lower levels of the intestine, nor of the quantity of the "neurohormones" liberated locally during the autonomic discharge, it is impossible to conjecture further into its physiological significance on any quantitative ground. By a mere inspection of the intestines, however, the duodenum is evidently more vascular than the ileum. It

seems that any substance carried to the muscle by the blood-stream, whether a stimulant or a depressant, would have a better chance to act on the duodenum. It is for this reason that we have not attempted to confirm our results by intravenous injection.

Summary.

1. The motility of the isolated rabbit intestine was registered in Tyrode solution. One, two, or three segments sampled from different levels of the intestine were used at a time and their response to various chemical stimulants and depressants compared.

2. Segments taken from upper levels of the intestine are generally more sensitive to acetylcholine, while those from lower levels are more senstitive to adrenaline.

3. This gradient of chemical excitability is easily upset by shock and asphyxia of the animal during the preparation of the intestine.

4. The stimulating action of barium, eserine, histamine, pilocarpine, and choline, and the depressing action of atropine, potassium, and magnesium are about equal in all the segments.

5. The difference in excitability of the intestinal muscle to the neuro-hormones is probably an innate property of the muscle fibres of the intestine which is closely related to the gradient of rhythmicity of the muscle. The possible physiological significance of the phenomenon is discussed.

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THE LOCALIZATION OF THE INTESTINAL INHIBITORY REFLEX ARC. By PEI-YEN CHANG and FONG-YEN HSU.

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ONE of the most outstanding features of visceral sensation is perhaps the primitive character of the nerve connections with the central nervous system. Most of the visceral impulses do not seem to reach the higher centres of the brain; if they do, they only give rise to a sensation of very ill-defined nature. Hence if subjective sensations be used as a criterion of sensitivity of the receptor, the digestive tube may be said to be insensitive to many kinds of stimuli that are effective in arousing skin sensations.

The most effective and perhaps most physiological stimulus for this organ is the distension of its wall, which, if intense enough, may be subjectively perceived as discomfort or pain [Kuntz, 1932]. Experimentally, various reflexes can be elicited by visceral stimulation. Distension of the gastro-intestinal tract is associated with an inhibition of the peristaltic movement [Pearcy and Van Liere, 1926; Youmans and Meek, 1937], dilatation of the pupil and fall of arterial blood pressure [Irving, McSwiney, and Suffolk, 1937], vaso-constriction of the toes and fingers [Carmichael *et al.*, 1939], and so on. Irving, McSwiney, and Suffolk [1937], working on the pupil-dilating reflex, found that while distension of the intestines was an effective stimulus, cutting, burning, or scraping the intestines gave rise to no response. On the other hand, it is also a familiar fact that mere handling of the exposed intestine is sufficient to cause a profound reflex inhibition of the whole length of intestine. Apparently most of the intestinal afferent impulses, though unable to reach the brain, can still call forth responses of the intestine itself through a shorter reflex arc. Indeed it has been shown that intestinal inhibition from distension can occur in the absence of extrinsic nerves [Youmans, Meek, and Herrin, 1938] and this is ascribed to enteric conduction. In the present investigation, only the reflex in its true sense has been studied. As will be seen later, the inhibitory reflex is elicitable by a variety of stimuli applied directly to the organ itself, or through its afferent nerves, and is perhaps a function of the spinal sympathetic centres. The mode of fibre connections concerned

seems that any substance carried to the muscle by the blood-stream, whether a stimulant or a depressant, would have a better chance to act on the duodenum. It is for this reason that we have not attempted to confirm our results by intravenous injection.

Summary.

1. The motility of the isolated rabbit intestine was registered in Tyrode solution. One, two, or three segments sampled from different levels of the intestine were used at a time and their response to various chemical stimulants and depressants compared.

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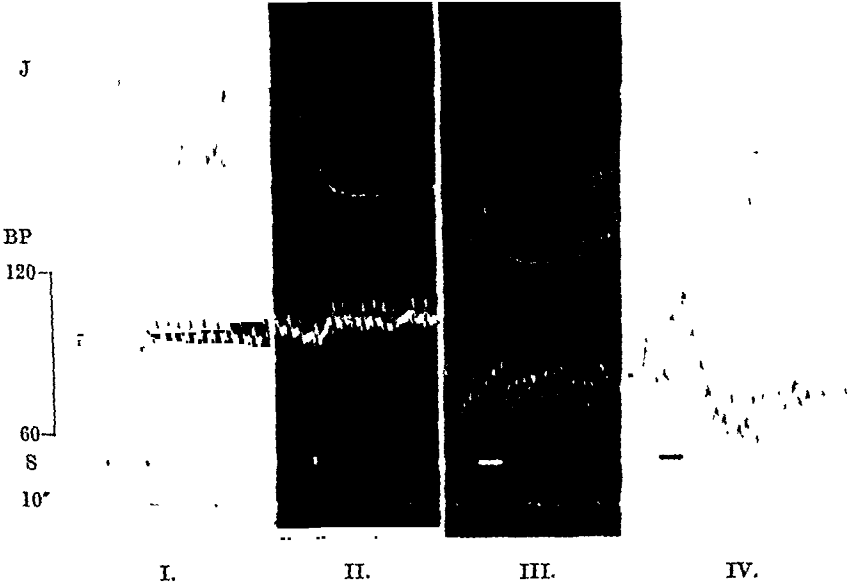

Fig. 1.—Dog. Morphine-ether-chloralose anæsthesia. J., motility of jejunum; B.P., blood pressure. I. Another loop of intestine distended with warm saline at 30 mm. Hg. II. Manipulation of another loop of intestine with hand. III. Stimulation of the central end of the divided mesenteric nerve. Coil distance at 9 cm. IV. Stimulation of the central end of the right vagus. Coil distance 5 cm.

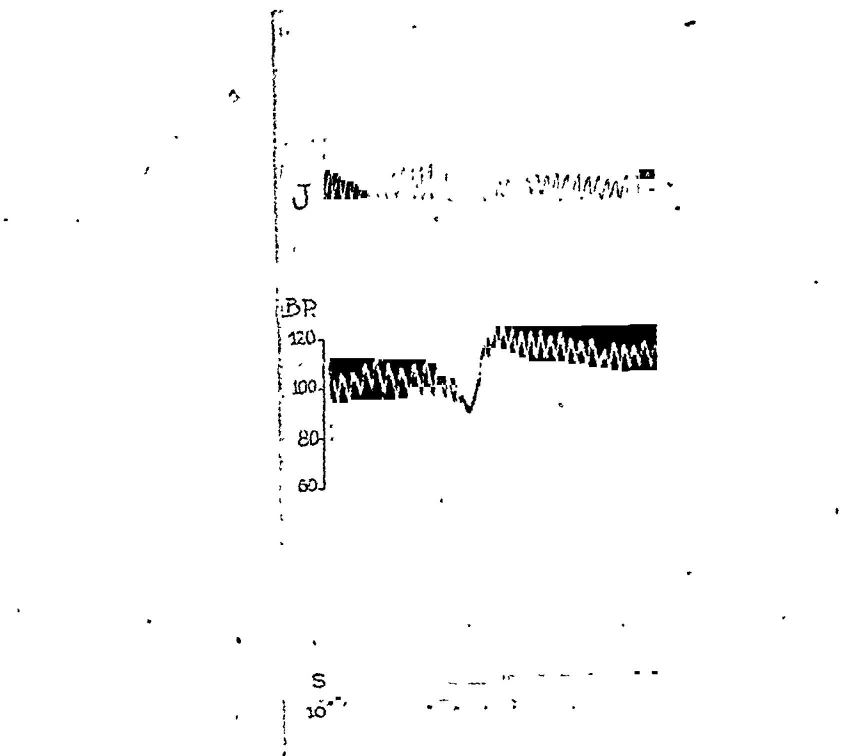

Fig. 2.—Dog. Morphine-ether-chloralose anæsthesia. Double adrenalectomy. Jejunum motility. At signal another loop was flushed inside with saline at 54° C.

in the local sympathetic reflex has further been investigated and will also be presented in this paper.

Experimental Method.

Adult dogs of both sexes were used. They were anæsthetised with ether after which chloralose (0·05 g. per kg.) was injected slowly into the saphenous vein. In most experiments 4 mg. morphine per kg. was given subcutaneously half an hour beforehand in order to enhance intestinal motility. After the insertion of tracheal and arterial cannulæ the abdomen was opened, and the motility of a desired segment of the small intestine was registered by the balloon method. Sometimes two balloons were used in order to record the motility of two segments simultaneously. Another loop of intestine was then pulled out and isolated from the rest by stout ligatures. It was then either connected through rubber tubing to a syringe for varying the intra-intestinal pressure or so arranged that saline of desired temperature and composition could be run through the lumen without at the same time affecting the pressure. Care was taken not to disturb the intestine during the application of the desired stimulus. In most experiments, however, the mesenteric nerve from a loop of intestine was also severed and its central end stimulated by the tetanic current, using a Du Bois Reymond inductorium (Palmer) with 8 volts in the primary. This mode of stimulation had the obvious advantage of being under quantitative control, though with no reference as to what sensory fibres are stimulated.

Results.

After administration of morphine the segmentation movements of the intestine were usually very active and regular during the early part of the experiment, if anæsthesia was deep enough and the intestine, if exposed, was kept warm and moist. When another loop of small intestine was handled, cut with scissors, distended with air or saline, or flushed with hot saline above 50° C., segmentation movements immediately diminished in size or entirely disappeared (figs. 1 and 2). The stimulation of the central end of the mesenteric nerve produced the same effects. On occasion we used several balloons to record the motility of several loops of intestine simultaneously. We found that the inhibition occurred with the same intensity from the lower part of duodenum to the ileum. Coincident with the intestinal inhibition the arterial blood pressure was also raised.

However, if intestinal movement was poor, as was usually the case when no morphine was given beforehand, no further inhibition could be produced by sensory nerve stimulation. In fact, a quiescent intestine might develop peristaltic contractions on afferent mesenteric stimulation. This is more so with the ileocecal sphincter. On the other hand,

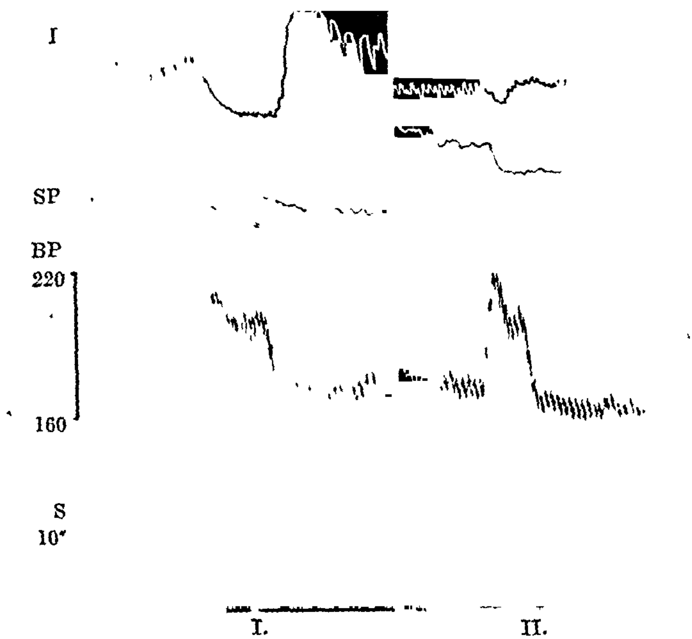

FIG. 3.—Dog. Ether-chloralose anæsthesia. Record from above downwards: motility of ileum, of I–C sphincter, blood pressure, signal for stimulation of central end of mesenteric nerve, time in 10 seconds. Between I. and II. 2 mg. morphine per kg. was injected subcutaneously.

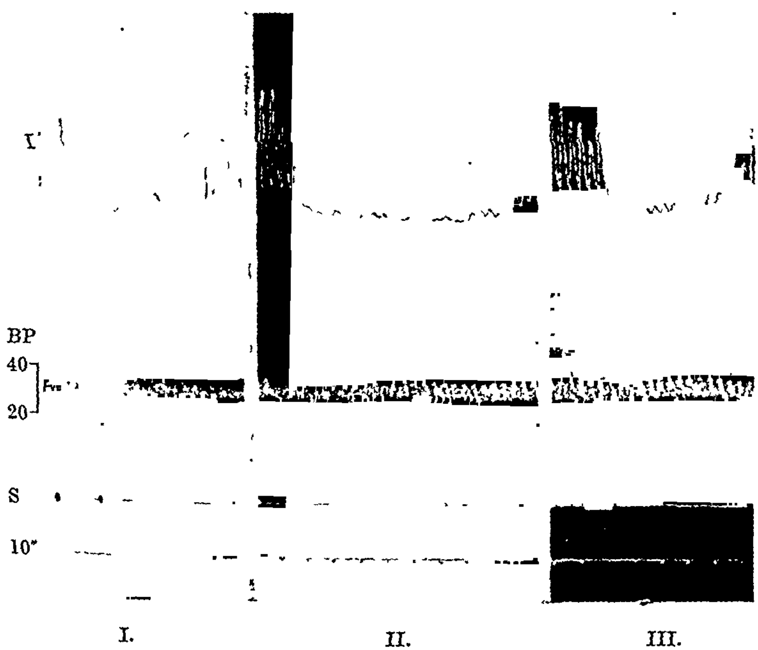

FIG. 4.—Dog. Morphine-ether-chloralose anæsthesia. Spinal cord transected at first thoracic. I. Intestinal motility. Another loop of intestine was distended at I. and manipulated at II. Stimulation of central end of mesenteric nerve at III. Coil distance 9 cm.

if the intestinal movement was active but the ileocecal sphincter was quiescent, the stimulation of the central end of a mesenteric nerve always caused reflex inhibition of the intestine but contraction of the sphincter. After the administration of morphine or barium chloride, which increased the tone of the sphincter, the reflex response of the latter to mesenteric afferent stimulation also changed into inhibition (fig. 3). This change seems to depend solely on the muscular tone and does not involve two different nerve pathways. For the present they will be collectively referred to as the intestinal inhibitory reflex.

We have also stimulated other afferent nerves of somatic as well as visceral origin: the vagus, the pelvic, and the saphenous or sciatic. Only stimulation of the central end of the pelvic nerve gave a result comparable with that of stimulation of the mesenterics. But the reflex excitability in the former case was much lower. The stimulation of the central stump of the saphenous or vagus commonly induced reflex augmentation of intestinal motility which persisted after section of both vagi. With very strong currents central stimulation of the vagus caused a delayed inhibition, which may be due to the discharge of adrenaline (fig. 1).

The intestinal inhibitory reflex persisted after bilateral vagotomy, adrenalectomy, and transection of the spinal cord at the upper thoracic level (fig. 4). However, it was abolished by cutting the splanchnic nerves. The evidence thus obtained indicates that the reflex centre lies in the spinal cord below the upper thoracic level and that either the afferent or efferent or both pathways are contained in the splanchnics. That the latter nerves contain visceral afferent fibres has already been abundantly demonstrated [*cf.* Bain, Irving, and McSwiney, 1935]. There is evidence that the abdominal vagus also contains such fibres. But according to Irving, McSwiney, and Suffolk [1937] the sensory innervation of the jejunum and ileum of the cat is mainly splanchnic. Our results on the dog have ruled out the vagus as an important sensory channel from the intestines, as the reflex remained unaltered after cutting the vagi. In all probability, therefore, both the afferent and efferent pathways are contained in the splanchnics.

The postganglionic station in the efferent path is situated in the cœliac and mesenteric plexus, since painting them with nicotine solution abolished the reflex completely.

The dorsal and ventral roots concerned in this reflex were explored by successive division from above downwards and from below upwards, the reflex being tested after cutting each pair. The results showed that the afferent paths enter the cord *via* dorsal roots between the seventh thoracic and first lumbar, and that the efferent paths leave the cord *via* ventral roots between the eighth thoracic and first lumbar. The last two thoracic roots seem to contain the greatest number of fibres to and from the small intestine since their section resulted in the

proper functioning of the isolated segments. This was accomplished by cutting the nerve tissue with silk ligatures around the cord and removing them shortly afterwards in order to re-establish blood circulation. In successful experiments the reflex remained either unaffected or only slightly diminished in size after the operation.

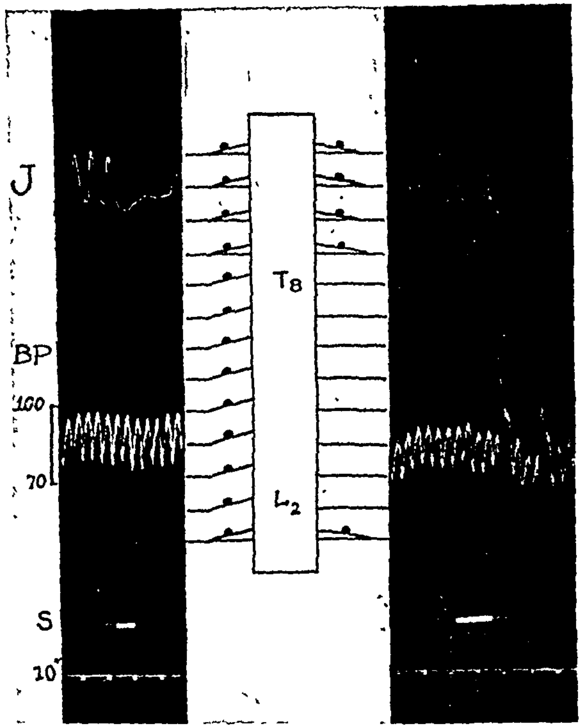

FIG. 6.—Dog. Morphine-ether-chloralose anæsthesia. Double vagotomy. Inhibitory reflex before and after dividing dorsal roots on the right side and ventral roots on the left side from T_8 to L_2. Mesenteric stimulation at signal. First stimulation: Coil distance, 9 cm. Second stimulation: Coil distance, 5 cm.

From the results presented above it can be concluded that most fibres of the afferent neurone of the local intestinal reflex arc are connected with the efferent neurone of the same side and same segment; relatively few cross to the opposite side or ascend or descend in the cord.

Discussion.

The intestinal reflex described in the present communication has both its afferent and efferent fibres in the splanchnic nerves, and is essentially a function of the spinal sympathetic centres, for both the inhibition of intestinal muscle and excitation of the ileocecal sphincter are signs of sympathetic discharge. The spinal roots concerned in the reflex as found by our methods are in no wise different from those

greatest loss of the reflex activity. No segmental difference was observed between the jejunum and ileum as regards both sensory and inhibitory fibres.

The question as to whether the reflex is a crossed one was attacked by cutting both dorsal and ventral roots on the same side in one set of experiments, and in another set the dorsal roots on one side and ventral

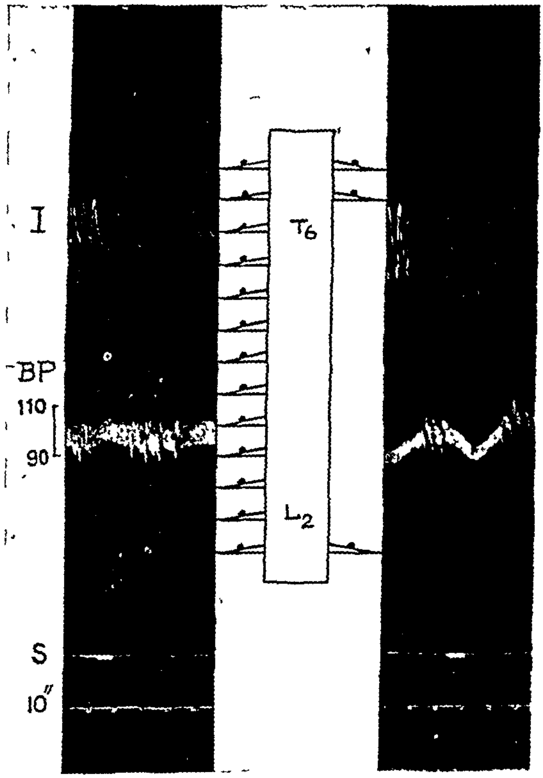

FIG. 5.—Dog. Morphine-ether-chloralose anæsthesia. Double vagotomy. Inhibitory reflex before and after cutting the spinal roots from T_6 to L_2 on the right side. Mesenteric nerve stimulation at signal. Coil distance, 9 cm. Stimulation time, 5 seconds.

roots on the other side were sectioned. The intensity of reflex activity was diminished by about one-half by the former procedure (fig. 5), but disappeared entirely by the latter (fig. 6). This suggests that the reflex is essentially an uncrossed one.

In another series of experiments the cord was divided transversely between each pair of spinal nerves from T_8 to L_2, so that the ascending and descending fibres in the cord were severed, but the reflex arc in each segment was intact. In these experiments it was essential that the blood supply of each segment must be kept intact in order to ensure

THE EFFECT OF ASCORBIC ACID (VITAMIN C), CALCIUM ASCORBATE, AND CALCIUM GLUCONATE ON THE REGENERATION OF BONE IN RATS.

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(*Received for publication 26th January 1942.*)

THERE is abundant evidence in the literature that vitamin C is associated with the production and maintenance of collagen fibres [Watanabe, 1924; Wolbach and Howe, 1925, 1926; Mazoué, 1937; Jéney and Törö, 1938; Quérido and Gaillard, 1939; Hunt, 1941].

Since bone possesses a fibrous collagenous-like matrix it is reasonable to expect an imperfect regeneration of bone in scorbutic animals. This has been shown to be a fact [Ferraris and Lewi, 1923; Israel and Frankel, 1926; Kappis, 1927; Schilozew, 1928; Salter and Aub, 1931; Roegholt, 1932; Jéney and Korpàssy, 1934; Hertz, 1936; Ham and Elliott, 1938; Klein, 1939].

This experimental work is supported by numerous clinical observations [*e.g.* Mead, 1762; Marrigues, 1783; Collisen, 1798; Hammick, 1830; Budd, 1840; Moore, 1859; Lobmayer, 1918, etc.].

The next question to be considered is whether the regeneration of bone can be accelerated by the administration of amounts of vitamin C in excess of that needed to saturate the organism with the vitamin. It seems unlikely that this would be so, because excess vitamin C is rapidly excreted in the urine.

Halàsz and Marx [1932] appear to have first studied this problem in guinea-pigs. They fed their animals on a diet which was rich in vitamin C, and in addition they administered lemon juice daily with a pipette. They found no radiographic or microscopic evidence that the healing of the fracture was accelerated. On the other hand, Lexer [1939] found that in three groups of guinea-pigs which received (i) a normal diet, (ii) a normal diet and extra vitamin C, and (iii) a diet deficient in vitamin C, the healing of fractures as determined by X-ray examination was best in group (ii) and worst in group (iii).

Hanke [1935] found that vitamin C injected into rabbits (which are usually regarded as being able to synthesise the vitamin and

described by previous workers. The more interesting point is the fact that sensory stimulation of the vagus and sciatic was not able to call forth the same response as mesenteric stimulation. This is perhaps contradictory to the statement that the small intestine undergoes inhibition on stimulating any afferent nerve. The nature of fibre connections in the cord further shows the primitiveness of this reflex, which is ipse-lateral and homosegmental. If analogy can be drawn at all between the visceral and somatic reflexes, this reflex is probably a counterpart of the myotatic reflex so far as the simplicity of the arc is concerned. However, while the latter reflex serves some purpose in maintaining posture of the body as a whole, the former reflex is probably merely protective in nature—this is, irritation of one part of the intestine prevents the passage of food through other parts so as to avoid over distension of the already congested gut.

Summary.

1. Stimulation of a loop of small intestine by pressure, heat, mechanical injury or electrical stimulation of its afferent nerve causes an inhibition of the whole intestine. It is a reflex response and is independent of the vagus and of the adrenal gland.

2. The reflex centre lies in the lower part of the thoracic and upper part of the lumbar cord. Afferent fibres enter the cord *via* dorsal roots from the seventh thoracic to the first lumbar segments. Efferent fibres leave the cord *via* ventral roots from the eighth thoracic to the first lumbar segments.

3. The fibre connections in the spinal cord was also investigated. It is found that most of the afferent fibres do not cross to the opposite side, nor ascend or descend in the cord, but make connections with the efferent neurones in the same side and same segment of the cord.

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It is difficult by these methods, however, to obtain fissures of the same size. It was decided in these experiments, in order to obtain exactly comparable areas for regeneration to take place, to use a dentist's twist drill of standard size and to bore in each case through the cortex of the bone into the medulla. Thus in a few minutes it was possible to obtain a series of lesions of identical size and shape.

The animals used in the present experiments were white rats of Glaxo, or Glaxo descended, stock, their body-weights varied between 70 and 130 g. Twenty-four hours before operation the hair from the whole of the hinder portion of the body, and of the outer surfaces of the hind legs, was removed with the aid of barium sulphide. Immediately prior to the operation the skin so exposed was painted with tincture of iodine or with a 1/1000 solution of acriflavine, and just before an incision was made the skin was washed with ether.

The rats were anæsthetised with nembutal, and were secured on a specially designed operating board which consisted of a rectangular block of wood fastened upon a flat board. The hind quarters of the animal were placed upon the block. The legs were secured by cords attached to two screws placed behind and to the side of the block. The thorax of the animal was held firmly to the flat board by a piece of cloth with drawing-pins. In such a position the animal was firmly held and the block acted as a support for the femurs whilst they were being drilled. Once secured on this board, the rat was placed in such a position that by making a skin incision in each leg, snipping through the subcutaneous connective tissue, and pushing the muscles to one side, the femur can be exposed. There was a negligible amount of bleeding at this stage. An electrically driven, 1 mm. diameter, dentist's twist drill was used to bore a hole approximately in the centre of the shaft of each femur. Penetration of the cortex of the bone resulted in a hæmorrhage from the marrow, but a small piece of cotton-wool left in contact with the hole for a few seconds after drilling stopped the bleeding. A single stitch of silk thread served to secure the fascia, and the skin wound was closed with one or two "Michel" clips, which were removed 3 days after the operation. The whole operation was performed using strict aseptic precautions, because, although the rat generally is not susceptible to pyogenic infections, bone does appear to be more easily infected than other rat tissues. In two cases slight suppuration of the skin wound was observed, probably due to post-operative infection, but in no case was any infection of the bone found to be present.

Immediately following the operation the animals were injected with the various test substances, and the injections were continued daily. Controls were given normal saline. After operation and first injection the animals, while still under the influence of nembutal, were wrapped in cotton-wool and placed together in a box with hot-water

therefore as being saturated with it) accelerated bone regeneration after resection. He found also that rabbits on a vitamin C free diet eventually regenerated resected bone, but did so only very slowly.

Giangrasso [1939 *a*] found that daily subcutaneous injections of 10 mg. of vitamin C into rabbits with a fractured forearm caused an increase in the rate of healing (radiographic and microscopic evidence). In another series of experiments [1939 *b*] he found injection of vitamin C more effective than injections of vitamin D. In a third paper [1939 *c*] he suggested that large doses of vitamin C (100 mg. a day) slightly retarded healing in the beginning, but that this action rapidly passed off.

None of the authors who have investigated the influence of *extra-saturation* vitamin C on fracture healing appear to have subjected their results to a statistical analysis, or indeed to have used methods which lent themselves to such an analysis, although Hanke has published some rather convincing X-ray photographs. An attempt has been made in the experiments recorded here to obtain an accurate estimation of the ability of various substances to accelerate bone regeneration.

Technique.

Varying techniques have been used for studying the regeneration of bone. In general, fracture of a bone, whether it be fracture of the femur [Israel and Frankel, 1926; Schilozew, 1928; Halàsz and Marx, 1932], fracture of the radius and/or ulna [Giangrasso, 1939], fracture of metatarsal bones [Otsuki, 1927; Emori, 1927], or fracture of the ribs [Moritsch and Krammer, 1929; Ferraris and Lewi, 1923; and Emori, 1927], is unsatisfactory for an accurate comparative study of the regeneration of bone. The reasons for this are that it is impossible to control the amount of trauma caused by each fracture, and if an injured limb has to be kept in splints or plaster to support it, and to keep the broken ends in apposition, these are rapidly gnawed off by the animals. Hertz [1936] has controlled most of these variables by the introduction of the fracture forceps and by its use to fracture the fibula of rats and guinea-pigs. One advantage of this method is that the leg does not require splinting, because the tibia supports the broken bone and the ends of the fractured bone are kept more or less in alignment by the surrounding soft tissues. Even so, it must be difficult to control adequately the trauma and the alignment of the broken ends, as well as the nature of the break.

Other workers have, by aseptic techniques, sawn through bones; and Vara Lopez [1928], in the case of an ulna treated in this way, rendered the two ends of the sawn bone immovable by suturing the limb into the muscles of the chest wall. Watanabe [1924] sawed a small fissure in the cranium, and Wolbach and Howe [1925, 1926] used a small circular saw to produce fissures in the femur of the guinea-pig.

At the conclusion of the experimental period the animals were killed with chloroform, the femurs were rapidly dissected out and fixed in 10 per cent. acetic acid containing 10 per cent. silver nitrate in dark brown bottles for 12 hours. This reagent was used because it was hoped to use them later for a cytological study of the distribution of vitamin C in the callus. After 12 hours the femurs were removed from the fixative, washed for an hour in distilled water, and then placed in 10 per cent. trichloracetic acid for 36 hours in order to decalcify them. They were then washed, dehydrated, and embedded in wax for standard periods of time. The use of standard times for these processes is essential, otherwise one gets variable shrinkage of the trabeculæ which have formed. In addition, all manipulation of the femurs from fixing to embedding was carried out in standard-sized bottles, one femur to each bottle, and a standard volume of fluid was used in each case. Serial sections were made through the hole which had been bored in the femur and the sections were stained with hæmatoxylin and van Gieson stains.

The work recorded in this paper deals with only one phase of the healing process, namely, the formation of temporary membrane bone. No attempt has been made as yet to investigate the effects of any of the substances injected on the formation of cartilage, on endochondral ossification, or on the subsequent remoulding process. Observation of the healing process at various times after the boring of the hole were made in order to ascertain when would be the most suitable time to estimate the degree of healing which had taken place. The amount of bony regeneration which had occurred in such a hole was followed by killing animals at 3 days, 4 days, 7 days, 11 days, 14 days, and 18 days after the operation. Immediately the hole has been bored there is a hæmorrhage from the medulla. The blood, however, clots in a few seconds. Two days after the operation, numerous fibroblasts are present in the clot and fine fibres ramify through it. These fibres do not stain with van Gieson and are probably composed of precollagen. By 4 days, strands of van Gieson staining material make their appearance, and finally appear to form themselves into anastomosing and branching trabeculæ. This stage has been reached by 7 days. This van Gieson staining material is ossein; it is the equivalent, in bone, of connective tissue collagen. Between 7 days and 18 days (the latest stage of healing investigated in this work) the changes consist chiefly of the further growing over and thickening of the periosteum and the development of cartilage in the callus. Some specimens show what appears to be cartilage formed by the periosteum at 4 days, but it is not until very late (*e.g.* around 18 days) that any appreciable amount of cartilage appears in the callus.

Since by 7 days an appreciable number of bony trabeculæ had been formed in the region of the hole, this time was taken as the

bottles until the next morning. They were given only water to drink during this period. The following morning they were placed, each in a separate cage. Each cage was of identical size, so that all the rats were able to take the same amount of exercise. Each animal received as much as it could eat daily of rat cake, milk, greens, and carrots, or, if these last two were not available, of dried alfalfa. Two drops of halibut liver oil were also given daily to each rat. Weighings of the amounts of food eaten by each rat showed that they consumed approximately the same amount of the various substances given, and it was therefore assumed that the dietary intake of calcium was approximately the same in all animals.

Usually five animals were operated on at the one time and each was given a different treatment. One was injected daily with vitamin C, one with calcium glucono-galacto-gluconate, a third with calcium ascorbate (a calcium salt of vitamin C), a fourth, in some experiments only, with separate solutions of vitamin C and calcium glucono-galacto-gluconate; and a fifth with normal saline. By distributing the animals of each series operated on throughout the experimental groups, any uncontrolled, or uncontrollable, variations which occurred at the time and which might have weighted the results towards any particular group were prevented.

All test substances were injected subcutaneously.

The following are the body-weights of the animals of the various experimental groups. There is no significant difference between any of them.

BODY-WEIGHTS.

Controls.	*Ca ascorbate.*	*Ca gluconate.*	*Vitamin C.*	Vitamin C and Ca gluconate.
$85{\cdot}8 \pm 8{\cdot}0$	$93{\cdot}0 \pm 4{\cdot}5$	$85{\cdot}3 \pm 8{\cdot}2$	$99{\cdot}0 \pm 9{\cdot}6$	97·0 (three animals only)

The Ca ascorbate group received 50 mg. of Ca ascorbate a day. The vitamin C group received the same amount of vitamin C as was contained in 50 mg. of Ca ascorbate, *i.e.* about 45 mg. The Ca glucono-galacto-gluconate group received an amount of calcium equal to that present in the calcium ascorbate, namely, about 5 mg. The group in which vitamin C and calcium glucono-galacto-gluconate and vitamin C were injected in separate solutions received the same amounts of these substances as were contained in the Ca ascorbate—that is, 45 mg. of vitamin C and 5 mg. of calcium.

Most of the animals used for this work were kept for 1 week after the operation. Other experiments were performed in which the animals were kept for 3 and for 4 days.

conception of an organic matrix being first laid down by regenerating bone, and this being later followed by the deposition of bone salts, is incorrect. According to them the organic matrix and the bone salt are laid down simultaneously, and if there is a delay in the deposition of bone salt it is an abnormality, and is "almost to be classed as a minimal form of rickets." Urist and McLean found that it occurred in some litter mates on the same diet and not in others. They suggest that it is due to an "insufficient local supply of bone minerals." Since vitamin C plays an important part in the formation of organic bone matrix and since calcium is an essential constituent of bone salt, it was considered that calcium ascorbate would be worth trying as an accelerator of bone healing. Secondly, the work of Ruskin [1938] suggested that calcium ascorbate was an ideal form in which to administer calcium. He claims that calcium gluconate is only 3 per cent. soluble in water and that Ca ascorbate is almost 100 per cent. soluble. The Ca ion content of a 3 per cent. solution of Ca gluconate he found to be 0·0014, and that of a 30 per cent. solution of Ca ascorbate was found to be 0·0226. This greater degree of ionisation suggested that Ca ascorbate would be more easily absorbed by the body tissues.

To ensure that any effect of the Ca ascorbate on the healing process was due to the injection of Ca as the ascorbate, a small number of animals were injected at the same time with separate solutions of vitamin C and Ca glucono-galacto-gluconate.

The compound calcium glucono-galacto-gluconate is produced by Sandoz and is a product which has improved solubility over the older Ca gluconate. The new product can, according to the makers, be obtained in a 30 per cent. solution. No Ca ion content for the various concentrations is published.

Results.

The following tables summarise the results obtained in this work. The trabecular index shown is an average figure obtained from five sections taken through the central region of the hole in each femur. That is to say, each index represents the average of ten samples.

Trabecular Indices.

Controls. Normal saline.	Ca ascorbate.
0·40	0·54
0·47	0·54
0·45	0·52
0·47	0·55
0·33	0·57
0·32	0·55
0·38	0·49
0·42	0·46
0·38	0·51
Mean $=0·402 \pm 0·0185$ $n=9$	Mean $=0·526 \pm 0·0115$ $n=9$

most suitable for killing the animals. An estimate of the amount of trabeculæ formed was obtained in the following way.

The slide bearing the sections through the hole in the femur was placed in a projector and the images of the trabeculæ (magnification ×100) present in a standard rectangular area were traced on to paper (see fig. 1). This was repeated in five separate sections of each femur, each section being taken at random near, or at, the centre of the hole. The standard rectangle of paper on which the trabeculæ had been traced

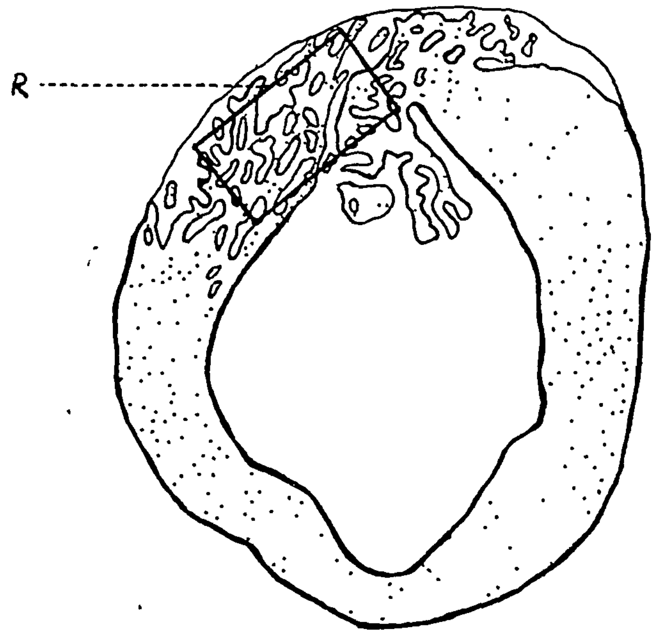

FIG 1.—Projection drawing (×32) of a transverse section through the femur of a rat. The section passes through the centre of a hole which has been drilled one week before. The rectangle of paper ("R") which includes the trabeculæ is weighed. The trabeculæ are cut out and weighed. The second weight divided by the first gives the "trabecular index."

was then weighed. Let its weight equal W. With a pair of curved scissors the trabeculæ were then cut out and weighed (let weight equal T). Then T/W gives an index of the amount of bone regenerated in the hole. This may be described as the "trabecular index" of regeneration. By means of this method it has been possible to obtain a series of figures which could be subjected to statistical examination.

Since rats synthesise their own vitamin C and can therefore be assumed to be saturated with the vitamin, they were therefore regarded as ideal animals for testing out by this method the effect of extra-saturation vitamin C on regeneration of bone.

Calcium ascorbate was tried as an accelerator for two reasons. In the first place, it has been shown by Urist and McLean [1941] that the

This is less than half the amount of bone formed by the ascorbic acid group, and only a little more than half the amount formed by the controls. It is difficult to imagine that these two substances injected in separate solutions into the same animal really do retard bone healing. Nevertheless the three results represent those obtained in three separate experiments. They were all rats which had been members of separate groups of five which had been operated on at the same time and injected with different substances; the results given by the other members of the three groups are shown below for comparison:

Vitamin C and Ca g.-g.-gluconate.	Ca ascorbate.	Vitamin C.	Ca g.-g.-gluconate.	Control. N. saline.
0·25	0·54	0·46	0·47	0·45
0·27	0·55	0·36	0·46	0·47
0·15	0·52	0·52	0·44	0·47

It is obvious that this matter needs further investigation.

Attempts have been made to estimate the effects of calcium ascorbate at earlier stages of the healing process, that is at 3 days and at 4 days. Four animals were injected with normal saline as controls and four were injected with calcium ascorbate for the 3-day experiments. The results are tabulated below:

THREE DAYS. TRABECULAR INDEX.

	Controls.		Ca ascorbate.
	0·066		0·066
	0·026		0·077
	0·057		0·023
	0·071		0·065
Mean	0·055 ± ·010	Mean	0·058 ± ·011

There is obviously no significant difference between these results.

Four animals were used in the 4-day experiment, two controls injected with normal saline, and two animals injected with calcium ascorbate.

FOUR DAYS. TRABECULAR INDEX.

	Controls.		Ca ascorbate.
	0·097		0·059
	0·032		0·060
Mean	0·065	Mean	0·060

This result is obviously not significant.

The results of the 3- and 4-day experiments (although from only a small number of animals) suggest that if Ca ascorbate has an effect

TRABECULAR INDICES—*Continued.*

Ca g.-g.-gluconate.	Vitamin C.
0·47	0·46
0·44	0·36
0·46	0·50
0·44	0·52
0·44	0·47
0·47	0·37
Mean $= 0{\cdot}453 \pm 0{\cdot}0069$ $n = 6$	Mean $= 0{\cdot}447 \pm 0{\cdot}0273$ $n = 6$

The following table summarises the results of the application of Fisher's "t" test for significance to these figures:—

	Ca ascorbate.	Vitamin C.	Ca g.-g.-gluconate.
Controls	$t = 5{\cdot}698$ P < 0·01	$t = 1{\cdot}423$ P lies between 0·2 and 0·1	$t = 2{\cdot}164$ P lies between 0·05 and 0·02
Ca ascorbate		$t = 3{\cdot}046$ P < 0·01	$t = 4{\cdot}807$ P < 0·01
Vitamin C			$t = 0{\cdot}2147$ P lies between 0·9 and 0·8

These results indicate that calcium ascorbate produces significantly more bone than that formed in the controls or in those animals which were injected with vitamin C or Ca g.-g.-gluconate. The amount of bone formed in the vitamin C injected group does not differ significantly from that produced by the controls or the calcium g.-g.-gluconate injected group. The latter group produces more bone than the controls, and the amount formed approaches a significant difference. Possibly if there were a greater number of animals in this group the difference may become significant. It is interesting that the calcium ascorbate group have produced more than the Ca g.-g.-gluconate group, because gluconic acid, although without antiscorbutic power, is closely related chemically to ascorbic acid.

Three animals were injected with Ca g.-g.-gluconate and vitamin C in separate solutions, and they gave the following results:—

TRABECULAR INDEX.

0·25
0·27
0·15
Mean 0·223

absorption may be vitamin C, although Henry and Kon [1939] found that rats on a diet adequate in phosphorus, but low in calcium, did not store more calcium if 2 mg. of vitamin C were added daily to the basal diet. Ruskin, however, appears to believe that the formation of calcium ascorbate in the small intestine is a preliminary to the absorption of calcium. He has pointed out that vitamin C dissolves calcium carbonate with a rapidity second only to fairly strong HCl. Ca ascorbate itself is a yellowish white amorphous powder which, according to Ruskin, is hygroscopic. One important point about calcium ascorbate is that the double bond structure of ascorbic acid is unaffected by the attachment of calcium to the vitamin C molecule, and the vitamin therefore retains its reducing power, and hence its biological activity.

Calcium ascorbate has a greater solubility and possibly a greater degree of ionisation than even the soluble glucono-galacto-gluconate of calcium. Therefore the present results may be due in part to the greater ease with which the calcium is absorbed from the subcutaneous tissues when the ascorbate is injected.

The availability of calcium to the body appears to some extent to be dependent upon how much of it is attached to serum proteins. Blood serum contains about three times as much calcium as can be held in an inorganic solution, due to the fact that an appreciable portion of the calcium is attached to the serum proteins. Ruskin and Jonnard [1938] showed, by means of a refractometer, that if calcium is added to blood as the ascorbate, the proteins fixed 0·8 mg. per c.c. On the other hand, if the calcium is added as calcium chloride, only about 0·4 to 0·5 mg. per c.c. of calcium is fixed by the proteins. If calcium gluconate is used, only 0·2 mg. is fixed.

The evidence available suggests, therefore, that Ca ascorbate is better ionised, more easily absorbed, and results in a higher concentration of calcium in the blood than other salts of calcium. This indicates that it may be an ideal form for therapeutic injection of calcium salts. Support for this contention is given by Ruskin, who claims that it is non-irritating by injection whether administered subcutaneously, intramuscularly, or intravenously, and that it is well tolerated by the gastro-intestinal tract. The same cannot be said of other calcium salts. For example, Aub [1937] points out that two salts of calcium may be used for injection—calcium chloride and calcium gluconate. The use of intravenous calcium chloride has numerous disadvantages, among which may be an occasional thrombosis of a vein, or, if some of it happens to be injected extravascularly, great pain and sloughing of the affected area are likely to result. Even intramuscular injections of calcium gluconate may cause vomiting in the patient, apart from what is described as the characteristic calcium effect of a feeling of great heat suffusing through the body. Aub points out that calcium salts

on the healing of fractures at a very early stage, the technique used is not delicate enough to detect it, or an insufficient number of animals was used in the experiments. Presumably, however, the effects of the Ca. ascorbate injections are cumulative, and after a week they are sufficiently advanced to enable a statistical difference to be detected.

It is possible that the injections of vitamin C, in the 7-day experiments, are slow in bringing about an effect, and that if the injections had been continued for a week longer a statistical increase in the amount of bone formed might have been seen in this group also. The literature, however, suggests an early effect of vitamin C injections. Giangrasso, in fact, claims that in rabbits vitamin C exerts an effect as early as 3 days after the first injection.

Discussion.

The results given in this paper show that vitamin C in the amounts injected does not accelerate the healing of bone in an animal such as the rat, which is normally saturated with this vitamin. They do show, however, that subcutaneous injections of calcium ascorbate are more effective than subcutaneous injections of either vitamin C or of calcium glucono-galacto-gluconate in accelerating the regeneration of bone in rats. These experiments do not of themselves give any indication whether subcutaneous injection of calcium ascorbate is better in this respect than simply feeding the rats with other calcium compounds or whether it is better than any of these compounds injected intravenously.

It is of interest, however, that, according to Shohl [1939], calcium is present in serum in a concentration of only 10·0 to 10·5 mg. per 100 c.c. of serum, and that unless an animal has been on a calcium deficient diet the ingestion of calcium salts produces only a minute rise in serum calcium. This rise is so small that for some time it was thought not to be significant.

On the other hand, Bell, Cuthbertson, and Orr [1941] stated that a daily consumption by growing rats of dietary calcium up to ·36 g. of calcium per 100 g. (dry weight) of food increased the weight, the calcium content, the bending and twisting strengths, and the thickness of the cortex, of their femora. Greater amounts of calcium had no effect. An accurate estimation of the calcium content of the diet fed to the rats used for this work has not been possible, but approximate calculations show that the percentage of calcium in their food approached the amount in the diet used by Bell *et al.*

Rats kept on a basal diet supplemented with orange juice have been shown by Lanford [1939] to absorb more calcium from the food than the control animals on the basal diet alone. The work of Ruskin [1938] suggests that the factor in orange juice which aids calcium

absorption may be vitamin C, although Henry and Kon [1939] found that rats on a diet adequate in phosphorus, but low in calcium, did not store more calcium if 2 mg. of vitamin C were added daily to the basal diet. Ruskin, however, appears to believe that the formation of calcium ascorbate in the small intestine is a preliminary to the absorption of calcium. He has pointed out that vitamin C dissolves calcium carbonate with a rapidity second only to fairly strong HCl. Ca ascorbate itself is a yellowish white amorphous powder which, according to Ruskin, is hygroscopic. One important point about calcium ascorbate is that the double bond structure of ascorbic acid is unaffected by the attachment of calcium to the vitamin C molecule, and the vitamin therefore retains its reducing power, and hence its biological activity.

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should always be injected very slowly, preferably over a period of not less than 5 minutes.

The amount of calcium injected into rats as ascorbate was equal to 5 mg. of Ca, and this was the equivalent of 3·5 g. of calcium injected into a 70-kilogram man. McCance and Widdowson [1939], however, found that the injection 0·186 g. of calcium intravenously was an uncomfortable experience, as it was accompanied by constant flushings and nausea. However, these authors injected the calcium as the gluconate, and it may prove possible to inject larger amounts of calcium with fewer unpleasant results if the ascorbate is used. This is a matter, however, for further investigation.

Summary.

A new technique has been described for the estimation of the power of a substance to accelerate the healing of bone.

It has been found by this method that neither vitamin C nor calcium glucono-galacto-gluconate in the doses given accelerates the healing of bone in rats on an adequate diet when injected subcutaneously.

On the other hand, it has been found that calcium ascorbate injected subcutaneously into rats does statistically increase the amount of bone regenerated by the end of 7 days.

The vitamin C used for these experiments was Redoxon, Roche, and I am greatly indebted to Roche Products Ltd., not only for the vitamin C, but also for making the calcium ascorbate specially for this work at my request.

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should always be injected very slowly, preferably over a period of not less than 5 minutes.

The amount of calcium injected into rats as ascorbate was equal to 5 mg. of Ca, and this was the equivalent of 3·5 g. of calcium injected into a 70-kilogram man. McCance and Widdowson [1939], however, found that the injection 0·186 g. of calcium intravenously was an uncomfortable experience, as it was accompanied by constant flushings and nausea. However, these authors injected the calcium as the gluconate, and it may prove possible to inject larger amounts of calcium with fewer unpleasant results if the ascorbate is used. This is a matter. however, for further investigation.

Summary.

A new technique has been described for the estimation of the power of a substance to accelerate the healing of bone.

It has been found by this method that neither vitamin C nor calcium glucono-galacto-gluconate in the doses given accelerates the healing of bone in rats on an adequate diet when injected subcutaneously.

On the other hand, it has been found that calcium ascorbate injected subcutaneously into rats does statistically increase the amount of bone regenerated by the end of 7 days.

The vitamin C used for these experiments was Redoxon, Roche, and I am greatly indebted to Roche Products Ltd., not only for the vitamin C, but also for making the calcium ascorbate specially for this work at my request.

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RENAL ARTERY LOOP IN THE DOG. By M. F. Lockett (Beit Memorial Research Fellow), W. J. O'Connor, and E. B. Verney. From the Pharmacology Laboratory, Cambridge.

(*Received for publication* 11*th March* 1942.)

We have been confronted with the necessity of securing accurate and enduring control over the blood-supply to the kidney in the living dog, and the method which we have finally found satisfactory—an adoption of that first described by van Leersum [1911] in connection with the carotid artery—is to enclose the left renal artery within a loop of skin. The deep position and short extent of the artery make it impossible to do this by transposing the kidney through the lumbo-dorsal fascia to the subcutaneous tissue of the loin: the skin must be taken down to the dorsal aspect of the artery before the formation of the renal artery loop is attempted.

The operation is done in two stages. Atropine sulphate, 2 mg., is given subcutaneously, anæsthesia induced with chloroform and ether, a tracheal tube passed *per os* and anæsthesia maintained with ether. The intubation of the trachea is a precautionary measure, taken because of the necessity of exposing the lower reflexion of the pleura during the subsequent dissection. The animal is laid on its right side and a small sandbag placed under the lumbar region. With full surgical precautions an incision is made through the skin and superficial fascia about 1 cm. to the right of the spinous processes, and extending from the level of the 13th thoracic spine above to that of the 6th lumbar below (see fig. 1). The skin is retracted to the left, the vertebral aponeurosis (A, fig. 1) divided longitudinally, the sacrospinalis group of muscles (B) is separated from the middle layer of lumbar fascia (C) and from the transverse processes (D) of the lumbar vertebræ, and the dorsal divisions (E) of the lumbar arteries and veins are divided between ligatures. The lateral two-thirds of the sacrospinalis muscles in the lumbar zone are now removed, and the transverse processes of the lumbar vertebræ, L2–L5 inclusive, resected subperiosteally. The ventral divisions of the lumbar vessels which are thereby exposed are divided between ligatures, and the psoas muscle (F), after separation from its fascia (G) and origins, is transected as it reaches the crest of the ilium and the anterior part removed. The dissection is now carried laterally over the dorsal aspect of the ventral layer of lumbar fascia and the transversalis fascia (H); and the quadratus lumborum (J) and the dorso-lateral parts of the oblique muscles (K, K′) are removed.

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By means of a series of silk sutures passed, on the one hand, through the deeper layers of the skin 2 to 3 cm. from its edge and, on the other, around the left crus of the diaphragm (L) and through the psoas fascia, the skin is fixed along and adjacent to the ventro-lateral surfaces of the

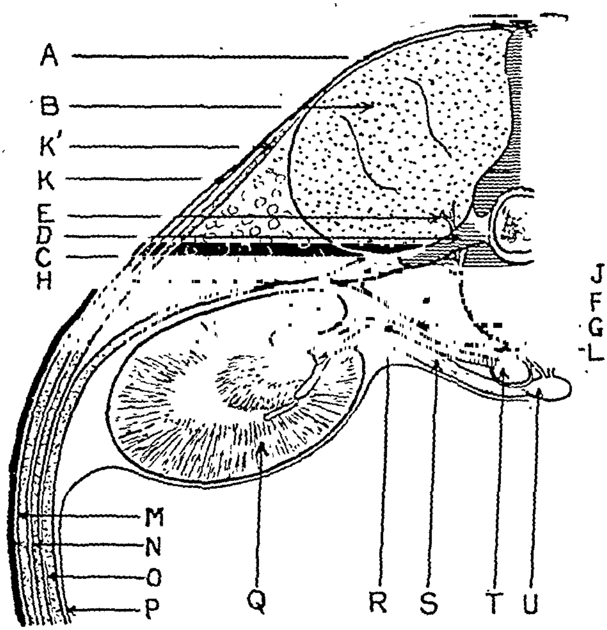

Fig. 1.—Diagrammatic transverse section of the posterior abdominal wall (left) at the level of the third lumbar vertebra in the dog. A, vertebral aponeurosis. B, sacrospinalis muscles. C, middle layer of lumbar fascia. D, transverse process. E, dorsal divisions of lumbar vessels. F, psoas muscle. G, psoas fascia. H, transversalis fascia. J, quadratus lumborum muscle. K, K', dorso-lateral parts of external and internal oblique muscles. L, left crus of diaphragm. M, N, O, external oblique, internal oblique, and transversus muscles. P, peritoneum. Q, kidney. R, renal artery. S, renal vein. T, aorta. U, inferior vena cava.

bodies of the lumbar vertebræ (fig. 2). The skin edges are then approximated by a series of silkworm-gut sutures (W). This completes the first stage, the renal artery (R) now lying undisturbed immediately beneath the skin and the thin layer of psoas fascia (fig. 2).

Some weeks later, at a time when the skin over the floor of the concavity in the left flank has become freely mobile on the superficial fascia, the second stage—the formation of the renal artery loop—is undertaken (see fig. 3). Again under ether anæsthesia and with full surgical precautions an incision about 5 cm. long is made through the skin, superficial and deep fasciæ, beginning some half-centimetre anterior to the origin of the renal artery and passing in a ventro-posterior direction parallel to the artery's course. The kidney is separated from the peritoneum, the renal artery isolated, and the kidney is slipped into a pocket between the skin and the lumbo-dorsal fascia (fig. 3). During

the isolation of the artery the renal nerves may be divided or no as desired; if the latter, the renal artery is carefully dissected from the surrounding nerves and these are left in company with the renal vein. A second incision is now made through the skin parallel to and some

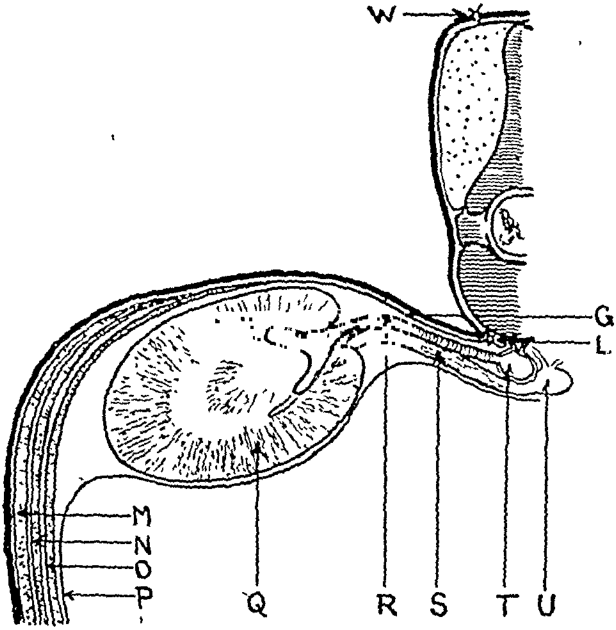

Fig. 2.—To show the completion of the first stage. G to U as in fig. 1, *q.v.* W, skin sutures.

1·5 cm. posterior to the dorsal half of the first incision. The two inner edges of skin are brought together beneath the renal artery by means of horse-hair sutures, and the two outer edges similarly sewn together under the tunnel of skin through which the renal artery now passes (V, V′). The suturing of the skin edges of the ventral half of the first incision completes the operation (fig. 3). In order to prevent the risk of the costal margin's pressing upon the upper pole of the kidney, we have found it desirable to resect the ventral halves of the lowest two ribs with their costal cartilages: this procedure may be incorporated in the first stage or separately undertaken between the first and the second stage. The exteriorized artery and the kidney are protected by an inner woollen jacket and an outer soft leather coat.

The health of the animals in which the renal artery has been exteriorized by the technique just described is excellent, and they suffer no obvious impairment of movement from the associated muscle loss. Fig. 4 is a photograph of one of our animals 7 weeks after the renal artery loop was made, and fig. 5 is a nearer view of the left lumbar region to show the loop and the outline of the left kidney.

Each of our animals has a denervated carotid loop [see Verney and Vogt, 1938] for measurement of the arterial pressure, and is perineotomized to facilitate the collection of urine by catheter and the

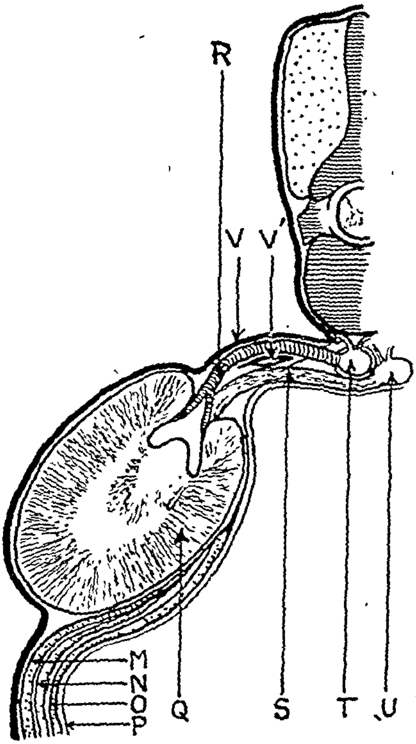

FIG. 3.—To show the completion of the second stage. M to U as in fig. 1, *q.v.* V, V′, skin loop containing renal artery.

measurement of its flow. The right kidney is removed at such time as the nature of the presenting problem prescribes.

SUMMARY.

A method is described whereby the renal artery in the dog is enclosed in a loop of skin, and the blood-supply to the kidney thereby brought under immediate external control.

Grateful acknowledgment is made to the Government Grant Committee of the Royal Society for defraying part of the expense incurred in this work.

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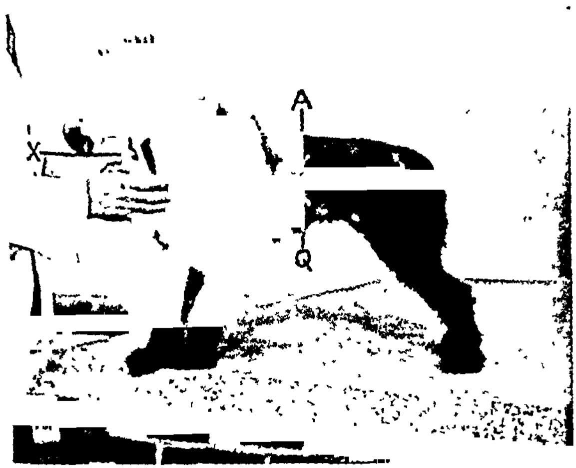

FIG. 4.—Photograph of a bitch 7 weeks after the renal artery loop had been made. A piece of whitened rubber tube (A) has been passed through the skin tunnel under the artery loop. Q, left kidney. X, denervated carotid loop, under which a small pad of wool has been placed.

FIG. 5.—Photograph of left lumbar region of the bitch shown in fig. 4. A, rubber tube passing beneath renal artery loop. Q, outline of left kidney.

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ACTION OF INTRATHECALLY INJECTED PROSTIGMINE, ACETYLCHOLINE, AND ESERINE ON THE CENTRAL NERVOUS SYSTEM IN MAN. By MICHAEL KREMER. From the Department of Medicine, British Post-Graduate Medical School, and Hammersmith Hospital, London, W. 2.

(*Received for publication 1st April 1942.*)

THE peripheral actions of acetylcholine and of various anticholine-esterases have been studied fully by many workers and are now well known. The action of these drugs on the central nervous system in mammals was first fully investigated by Schweitzer and Wright [1937, *b*, *c*, *d*; 1938] and Schweitzer, Stedman, and Wright [1938, 1939]. They demonstrated by a variety of experimental techniques that acetylcholine and other choline derivatives, and eserine, prostigmine, and other anticholine-esterases influenced reflexes by a direct action on the spinal cord. Only their main findings need be referred to here. In cats under chloralose anæsthesia acetylcholine depresses the knee jerk by a direct action on the spinal cord; sometimes an initial phase of stimulation was observed. Intravenously injected prostigmine and other so-called quaternary anticholine-esterases (*e.g.* eserine methiodide, dimethyl carbamic ester of hordenine methiodide) depress the knee jerk and other spinal reflexes (*e.g.* crossed extensor reflex, strychnine convulsions) by a direct action on the spinal cord. Intravenously injected eserine sulphate and other so-called tertiary anticholine-esterases (dimethyl carbamic ester of hordenine hydrochloride; methyl carbamic ester of *m*-hydroxyphenyl dimethyl ammonium hydrochloride) increase the knee jerk, and general reflex excitability, by a direct action on the central nervous system and may give rise to convulsions. The earlier work on this subject is fully reviewed in the papers by Schweitzer and Wright. More recently fresh observations have been reported by Bonvallet and Minz [1938], Merlis and Lawson [1939], Miller, Stavraky, and Woonton [1940], Chute, Feldberg, and Smyth [1940], Torda [1940], McKail, Abrador, and Wilson [1941], and Bülbring and Burn [1941]. All these workers agree with the main conclusion of Schweitzer and Wright that acetylcholine, eserine, and prostigmine have a direct action on the central nervous system. The exact details of the findings reported differ, however, in certain respects, depending to some extent

on species, experimental technique, and type of reflex studied. Some of these differences will be referred to in the discussion.

Little experimental work has been carried out on the action of these drugs in man. Henderson and Wilson [1936] injected eserine and acetylcholine into the lateral ventricle in man, and found that both drugs produced similar effects; these consisted of nausea, vomiting, increased intestinal movements and sweating, but the circulation and respiration were unaffected. The action of both drugs was abolished or prevented by atropine. Subthreshold doses of eserine potentiated the action of subsequently administered acetylcholine. Williams and Russell [1941] in epileptics found that prostigmine injected subcutaneously increased *petit mal* activity recorded in the electroencephalogram, while eserine generally reduced this activity. In a preliminary report, Kremer, Pearson, and Wright [1937] showed that the introduction of 1 mg. of prostigmine into the cerebro-spinal fluid by means of lumbar puncture in patients with hemiplegia decreases or abolishes tendon reflexes and muscle tone in the legs and sometimes in the arms too, without change in sensation. These investigations have been extended and form the basis of this paper.

Methods.

In all cases the substances used have been injected into the lumbar theca. Occasionally the lumbar puncture was preceded by an injection of atropine sulphate subcutaneously. The drugs used were solutions of prostigmine, eserine sulphate, and acetylcholine. These have been administered both to subjects with no abnormality of the central nervous system and to cases with some form of spasticity of pyramidal origin. The effects of both prostigmine and eserine sulphate have also been examined in cases of spinal block. The volume of the solution used never exceeded 2 c.c., and was always injected slowly and at room temperature. Occasionally a small quantity of cerebro-spinal fluid was withdrawn for analysis, but in most cases this was not done. The drug solution was mixed with the cerebro-spinal fluid withdrawn into the syringe, and the whole injected slowly. If a blood-stained tap was obtained, the experiment was postponed to another day.

Results.

Control Experiments.

Three patients were employed, one normal, one with cerebral thrombosis and spastic hemiplegia, and one with disseminated sclerosis. In each case the procedure was the same. An injection of 1 mg. of atropine sulphate was first given subcutaneously. Ten minutes later, lumbar puncture was performed and 2 c.c. of sterile distilled water were

injected. No effects were noted during the next three hours, after which no further observations were made. Two of these cases later received prostigmine and gave the typical results reported below.

Action of Acetylcholine alone.

Eight cases were used and eighteen experiments performed. Fresh solutions of acetylcholine in sterile distilled water were prepared immediately after the lumbar puncture was performed. This was done to ensure the minimum time interval between the making of the solution and the injection. The initial dose used was 2 mg. of acetylcholine. This was increased up to 500 mg., but no effects were noted in any system with any dose. Observations were made for three hours. The experiments were carried out in one normal subject, four cases of cerebral diplegia, two of spastic hemiplegia, and one of amyotrophic lateral sclerosis. All these cases responded to prostigmine in the manner reported below. In the last twelve experiments, no atropine was used as a preliminary medication, but the results were not changed in consequence.

Action of Prostigmine.

Prostigmine in doses varying from 0·1 mg. to 1·5 mg. was injected into the lumbar theca 73 times in 37 patients. The large number of cases examined is due to the fact that all the individuals recorded in this paper had an injection of prostigmine at some time to ensure that they gave the usual reaction to this substance. The cases used were:

Spastic hemiplegia	15
Disseminated sclerosis	12
Cerebral diplegia	6
Spinal block	2
Amyotrophic lateral sclerosis	2

Action of Prostigmine in Hemiplegia.

Two typical experiments in two cases of spastic hemiplegia will be described first.

Case 1.—J. B., male, aged 61, with hypertension and arteriosclerosis, had a residual left hemiplegia following a cerebral thrombosis 6 months previously. Examination revealed a considerable increase of tone in the antigravity muscles of the left arm and leg with a 50 per cent. loss of power. There was slight weakness of the left side of the face. All the deep reflexes of the left side were exaggerated. The abdominal reflexes could not be obtained; the left plantar response was "extensor," the right being "flexor." There was sustained left patellar

and left ankle clonus. Superficial, deep, and visceral sensation were unaffected.

Intramuscular and intravenous injections of prostigmine were given to this patient in doses up to 5 mg. The usual effects of muscular twitching, increased tone and reflexes on the spastic side, and abdominal pain were obtained in 15 minutes. There was no change in power or sensation. These effects persisted for 90 minutes and then a gradual return to normal was seen.

Effects of Intrathecal Injection of Prostigmine in Case J. B.

The initial pulse-rate was 88 per minute and the blood-pressure 210/115 mm. Hg.

10.10 a.m. Intrathecal injection of 1 mg. of prostigmine. The results obtained are summarised below and are set out in full in the accompanying tables.

10.20 a.m. Pulse 88. B.P. 210/115; no other change.

10.37 a.m. Pulse 100. B.P. 200/110; no other change.

10.50 a.m. Pulse 96. B.P. 195/115; face and arms unaffected.

Legs: Left ankle clonus now not sustained; diminution in left ankle jerk. Passive movements of left knee and ankle are easier. Power is unaffected.

10.58 a.m. B.P. 195/115. Diminution in both knee jerks. Both ankle jerks gone. All clonus disappeared. Power in left leg diminished.

11.15 a.m. All reflexes in both legs gone. Both plantar reflexes not obtained. Left leg is now flail-like. Arm reflexes are slightly diminished on both sides.

11.40 a.m. B.P. 200/115. Legs generally as before, but patient can now hardly lift the left leg from the bed. Arm reflexes are obtainable but much weaker. Tone in arms is less.

12.05 p.m. Pulse 84. B.P. 200/115. Legs as before. Arm reflexes all lost. Spasticity in left arm completely disappeared. Power in left arm much diminished. Patient is feeling weak, is pale, sweating and nauseated. Sensation unaffected.

12.45 p.m. Pulse 86. B.P. 210/115. No change in limbs. Following a cup of tea given at 12.30 p.m. the patient vomited. Is very pale, sweaty, and sleepy. The skin is cold. He wishes to pass urine, but cannot.

1.0 p.m. Given 1 mg. of atropine intravenously. No effect.

1.30 p.m. Pulse 84. B.P. 205/105. No change. Still wishes to pass urine, but cannot. Sleepy.

2.15 p.m. No change.

2.45 p.m. B.P. 240/120. Catheterised. Returning power in arms and reflexes in arms now obtained.

3.30 p.m. Returning power, tone and reflexes in legs.

4.30 p.m. Still some diminution of tone in legs, but otherwise the physical state has returned to pre-injection condition.

The effects of intrathecal injection of prostigmine in this patient are set out in Table I.

Comments.—Examination of the data shows the following points. The changes in blood-pressure, pulse-rate, and respiration were small and variable and bore no relationship to the changes in the nervous system. Symptoms began to appear after a latent period of 40 minutes and involved the left (hemiplegic) side before the normal side. The first changes noted were in the region of the body supplied by the most distal part of the spinal cord, indicated by decrease in the left ankle jerk and left ankle clonus, and some decrease in tone in the muscles round the left ankle and knee. The normal side of the body was soon involved and after 48 minutes both ankle jerks and the left ankle clonus had been abolished. A more proximal part of the body was now affected as shown by decrease in both knee jerks. At this stage some decrease in voluntary power on the left (hemiplegic) side occurred (from 50 per cent. to 25 per cent.), but none occurred on the normal side. After 65 minutes the left (hemiplegic) leg was completely toneless and all deep reflexes in both legs had disappeared. The plantar reflexes which had persisted hitherto, disappeared on both sides, the extensor response on the hemiplegic side simultaneously with the flexor reflex on the normal side. Only now, when leg reflex activity on both sides had almost vanished, did arm changes begin to appear. The change consisted of slight diminution of the deep arm reflexes. At 90 minutes power in the left (hemiplegic) leg had further declined to 10 per cent., and arm tone as well as deep reflexes on both sides showed a further decline; 20 minutes later, at 110 minutes, all arm reflexes had disappeared, all spasticity in the left (hemiplegic) arm had gone, and power in the weak, left, arm had further declined from 50 per cent. to 25 per cent. At this stage nausea set in, followed 40 minutes later (at 150 minutes) by vomiting, drowsiness, pallor, cold skin, and inability to pass urine. The state of power, tone, and reflexes remained unchanged for the next 65 minutes until at 215 minutes arm reflexes began to return more readily on the normal (right) side than on the hemiplegic (left) side, and 45 minutes later (260 minutes) recovery was proceeding rapidly, being further advanced in the arms, where it was almost complete, than in the legs; 60 minutes later (320 minutes) recovery was almost complete everywhere, and the general mental state was brighter and the skin circulation was normal. Sensation was unimpaired throughout. The face was not involved at any stage of the experiment.

The march of the symptoms up the leg from the distal to the proximal end (the plantar reflex being an exception), and then the

TABLE I.

	10.10 a.m.		10.20.	10.37.	10.50.	10.58.	11.15	11.40	12.05 p.m.	12.45	1.00.	1.30.
jerk. R. L.	+ + +	Injection 1 mg. prostigmine intrathecally.	No change	No change	+ + +	± ±	– –	– –	– –	– –	Given 1·5 mgm. atropine intravenously.	– –
e jerk R. L.	+ + +				+ +	– –	– –	– –	– –	– –		– –
lar clonus R. L.	– +				– +	– –	– –	– –	– –	– –		– –
e clonus R. L.	– +				– ±	– –	– –	– –	– –	– –		– –
ar reflex R. L.	"Flexor" "Extensor"				"Flexor" "Extensor"	"Flexor" "Extensor"	O O	O O	O O	O O		O O
R. L.	N + +				N +	N –	N –	N –	N –	N –		N –
r R. L.	100 50				100 50	100 25	100 25	100 10	100 10	100 10		75 10
ation R. L.	N N				N N	N N	N N	N N	N N	N N		N N
s jerk R. L.	+ + +				No change	No change	+ +	+ +	– –	– –		– –
ps jerk R. L.	+ + +						+ +	± ±	– –	– –		– –
R. L.	N + +						N +	N –	N –	N –		N –
r R. L.	100 50						100 50	100 25	100 25	100 25		100 10
tion R. L.	N N						N N	N N	N N	N N		N N
rate	88			100	96	92	90	88	84	86		80
-pressure	210/ 115		200/ 110	200/ 110	195/ 115	195/ 115	195/ 115	200/ 115	210/ 115	210/ 115		205/ 110
					Passive movements in ankle and knee feel freer.	Patient says arm feels freer, but no change observed.		Patient complains of feeling weak. Can hardly lift left leg from the bed.	Patient now appears pale and slight sweating seen. Complains of feeling sick.	Following a cup of tea given at 12.30, patient vomited. Looks very pale and sleepy. Sweating, skin cold. Wishes to pass urine (cannot).		Still wishes to pass urine. Still pale and sweating. Has vomited once again. Trying to sleep.

N: normal. O or –: response absent.

involvement of the arms, is striking and was a regular finding. During recovery in this case the arm returned to normal sooner than the leg, but this was not invariable. The general disturbance (drowsiness, vomiting), when they occurred usually set in when the depression of reflex and voluntary activity was most marked.

Case 2.—F. K. Male, age 65. Right-sided hemiplegia (due to cerebral thrombosis) 17 months. Initial clinical state:

Left side: Power, tone, and reflexes in legs, arms, and face normal.

Right side: Leg—Power weak; tone + +; ankle jerk +; ankle clonus + + and sustained; knee jerk + +; plantar response "extensor." Arms—Power weak; tone + +; all reflexes + +. Face—Slight weakness lower half of face; speech slightly affected.

Abdominal reflexes present in all four quadrants.

11.45 a.m. Inject 1/50 grain of atropine subcutaneously and 1 mg. of prostigmine intrathecally.

12 noon. (15 min.) No change.

The subsequent changes are fully set out in Table II.[1]

The detailed findings in case 2 resemble in all essential respects those reported in case 1. The latent period was 15 minutes before the first changes occurred, consisting of weakening of the ankle reflexes on the normal (left) side. At 55 minutes on the right (hemiplegic) side the extensor plantar response and ankle clonus had disappeared and both ankle and knee jerks, previously exaggerated, were subnormal. On the normal (left) side ankle and knee jerks had gone, but the flexor plantar response persisted. Tone was abolished in both legs and power on the left (hemiplegic) side had declined below its initial weak level. At this stage, when the involvement of the legs was so extensive, the arms were comparatively unaffected except for a decrease in tone in the originally spastic (left) hemiplegic arm. Involvement of higher levels was indicated by slight worsening of speech, and the state of drowsiness. At 120 minutes the facial involvement was greater and the general depression very marked. At 240 minutes all tone and reflexes in both arms were gone, all power in the right arm had disappeared, the facial weakness was marked, but the general condition (colour, alertness) had improved. At 275 minutes recovery was beginning in the right leg; at 330 minutes the left leg was normal and the right leg progressing further, and considerable arm recovery had taken place, especially on the left side. At 360 minutes a detailed study showed that recovery was proceeding generally, but was not especially more rapid in one part than another.

Again no change in sensation occurred at any stage of the experiment.

[1] A film showing the clinical findings at 12.15 and 12.45 p.m. was shown to the Physiological Society in 1937.

Table II.

		Initial.	11.45 a.m.	12.15 p.m.	12.40 p.m.	1.45 p.m.	3.45 p.m.	5.15 p.m.	5.45 p.m.	7
Knee jerk	R.	+ +	Inject 1/50th grain of atropine subcutaneously and 1 mg. of prostigmine intrathecally.	+ +	Weak	O	O	O	Weak	
	L.	Brisk		Diminished	O	O	O	O	O	
Ankle jerk	R.	+ +		+ +	Weak	O	O	Weak	Weak	
	L.	N		Diminished	O	O	O	O	O	
Ankle clonus	R.	+ + sustained		+ + sustained	O	O	O	+ sustained	+ sustained	s
	L.	O		O	O	O	O	O	O	
Plantar reflex	R.	Extensor		Extensor	O	O	O	Feeble flexor	Feeble flexor	I
	L.	Flexor		Flexor	Flexor	O	O	Flexor	Flexor	
Tone	R.	+ +		+ +	O	O	O	Slight	Quadriceps increased	
	L.	N		N	O	O	O	N	N	
Power	R.	Weak		Weak	Further diminished	O	O	Weak	Improving	A
	L.	N		N	N	N	N	N	N	i
Biceps jerk	R.	+ +		+ +	+ +	O	O	+	+	
	L.	Brisk		Brisk	Brisk	O	O	±	±	
Triceps jerk	R.	+ +		+ +	+ +	O	O	+	+	
	L.	Brisk		Brisk	Brisk	O	O	+	+	
Tone	R.	+ +		+ +	+	O	O	Triceps +	Triceps +	L
	L.	N		N	N	N	N	N	N	I
Power	R.	Weak		Weak	Weak	O	O	Wrist movements present	Elbow flexion present	Ir
	L.	N		N	N	N	N	N	N	
Abdominal reflexes	R. upper	+		+	Diminished	O				
	lower	+		+	O	O				
	L. upper	+		+	Diminished	O				
	lower	+		+	O	O				
Face	R.	Slight weakness, lower half		Slight weakness, lower half	Slight weakness, lower half	Right side puffs out with respiration	Very weak; not puffed out on respiration	Very weak	Very weak	
	L.	N		N	N	N	N	N		
Speech		Slightly affected		Slightly affected	More indistinct	Very indistinct	Very indistinct		Better	
Mental state		N		N	Drowsy and quiet	Very drowsy	Drowsy	More awake	Still more awake	B
Bladder		N		N	N	N	Distended, but cannot empty it	Still cannot pass urine	Catheterized, 20ozs. urine	
Colour		N		N	N	Intense pallor, face and neck	Less pale, face warmer		Better	
General state		Good		Good	Good	Vomited. Looks collapsed	Still feels sick		Improving	
Sensation		N		N	N	N	N	N	N	
Treatment						Inject 1/100 g. atropine, 4 c.c. coramine				

Next Morning.—Complete return to pre-injection state, but freer voluntary movements in right arm.
N: normal. O. absent.

All the experiments with prostigmine in spastic subjects have given the same results without any exception. There have been slight variations in quantitative effects, but never any variation in the qualitative nature of the response.

Typical effects were produced (though lasting only for one hour) with a dose of 0·1 mg. of prostigmine intrathecally. In this case, although the ankle jerk was abolished, a faint flicker of the knee jerk persisted throughout and the arm reflexes were obtained easily, though to a diminished degree. The effect on the tone of the various muscles was roughly proportional to the degree of diminution in reflexes. There was no vomiting, though the patient complained of nausea.

The average duration of the diminution in tone with an intrathecal dose of 1 mg. was about 6 hours. In only one case did it exceed 12 hours and actually lasted for 26 hours; no special reason could be found for this.

The contrast between the effects of prostigmine injected intramuscularly or intravenously on the one hand and intrathecally on the other is very striking. Injected by the former routes prostigmine produces its characteristic peripheral effects on viscera and on skeletal muscle, leading to increase in muscle tone and in reflexes and to some muscular twitching. Given intrathecally exactly the reverse effect on skeletal muscle was produced, namely, diminution or loss of muscle tone and reflexes. These changes must be attributed to a central action, as any prostigmine absorbed from the spinal theca into the general circulation would have had the opposite peripheral effect.

Action of Prostigmine in Cases of Spinal Block.

Two cases of spinal block were studied; both were due to carcinomatosis of the spinal column. The diagnosis of block was confirmed by lumbar puncture and by Queckenstedt's test. Typical results were obtained as shown in the following protocol:—

J. P. Clinically a case of spinal block at the level of lumbar 1 and 2. The legs were held in a flexor position with occasional painful flexor spasms; they were spastic, with exaggerated reflexes; the plantar responses were "extensor." There was slight analgesia below the level of the lesion and incontinence of urine. The abdominal reflexes were not obtained. The arms and face appeared normal in all respects.

25.11.38.

11.5 a.m. B.P. 140/80. Pulse 100. Injection of 0·5 mg. of prostigmine intrathecally below the level of the block.

11.30 a.m. B.P. 140/80. Slight diminution of spasticity of legs. No change in reflexes.

11.55 a.m. Leg reflexes now definitely diminished on both sides.

12.15 p.m. Loss of tone in all leg muscles on both sides. All leg reflexes have disappeared except for the plantars which showed a slight flicker. Sensation unaffected. B.P. 135/80. No flexor spasms now occur, even when the legs are scratched. Patient says that the legs feel much easier now that the "cramps" have gone.

12.50 p.m. As above, except that the plantar reflexes have disappeared.

1.50. No change. Patient took a light lunch. No nausea or vomiting. Arms show no change from pre-injection state.

2.50 p.m. B.P. 135/80. Patient is sleeping; no pallor or sweating. No change in condition.

7.0 p.m. Ankle jerks just obtained, but no obvious return in leg muscle tone.

26.11.38.

10.0 a.m. As in pre-injection condition, but flexor spasms had only returned on waking that morning.

The flexor spasms and the "extensor" plantar response found in this case prior to the injection correspond to the "flexor reflex" of lower mammals. Attention is especially drawn to the fact that the flexor spasms, the extensor plantar response, and the flexor posture in the legs were all abolished by the intrathecal injection of prostigmine below the level of the spinal block. No changes were noted in the muscles supplied by the spinal cord above the level of the block.

Action of Prostigmine in Subjects with Normal Spinal Cord.

Prostigmine was injected intrathecally (dose 1 mg.) in three individuals in whom (as far as could be determined) the spinal cord was normal. The results obtained were the same as in the spastic cases, *i.e.* loss of reflexes, vomiting, pallor, and sweating. There was no change in sensation. In two cases there was loss of ability to empty the bladder. The effect on tone could not satisfactorily be assessed, but power in the hands was measured by a dynamometer, with the following results in one case. The patient was asked to exercise the maximum grip possible every time.

10.0 a.m. Intrathecal injection of prostigmine, 1 mg.

	Hand Grips. R.	*Hand Grips.* L.	
10.0	250	220	
10.30	250	220	
11.0	230	210	
11.30	200	176	
12.0	150	140	
12.30	100	100	Vomited
1.0	100	110	Vomited
2.0	120	120	
3.0	120	120	
4.0	190	180	

The greatest loss in the power of the hand grips occurred during the time of loss of the arm reflexes. It also coincided, however, with the period of vomiting. It should be noted though that considerable loss of power occurred before the vomiting began, and indeed before there was any nausea.

Action of Acetylcholine together with Prostigmine.

In one case of disseminated sclerosis, the effect of intrathecal acetylcholine was tried after intravenous injection of prostigmine. 5 mg. of prostigmine were injected intravenously and, half an hour later, when the usual peripheral effects of prostigmine were well marked, 10 mg. of acetylcholine were given intrathecally. No effects were noticed during a three-hour period of observation.

In two cases the effects of a combined intrathecal injection of 0·1 mg. prostigmine and 10 mg. of acetylcholine were studied. The effects of injecting each substance alone by the same route was previously determined. No changes occurred after the injection of acetylcholine alone; prostigmine given alone had the small effect referred to above, *i.e.* abolition of the ankle jerk, diminution of the knee jerk, and decrease in muscle tone. The effects of the combined injection are set out below:—

S. S., a case of disseminated sclerosis with optic atrophy, nystagmus, precipitate micturition, marked spastic paresis of both arms and legs, exaggerated deep reflexes, ankle clonus.

10.40 a.m. B.P. 140/90. Pulse 84. Injection of 0·1 mg. of prostigmine and 10 mg. of acetylcholine intrathecally.

11.0 a.m. No change.

11.15 a.m. B.P. 135/95. Ankle clonus now reduced to one or two kicks only. Slight diminution of tone in muscles round ankle joints. Ankle jerk diminished.

11.30 a.m. No ankle clonus. Ankle jerks doubtful. Knee jerks present, but much diminished. Tone diminished. Sensation unaffected. Power appears unaffected.

11.45 a.m. B.P. 130/90. Knee and ankle jerks absent. Tone in legs absent. Sensation normal. Arms unaffected.

12.15 p.m. B.P. 130/90. Legs as above; slight diminution of tone in arms.

12.30 p.m. B.P. 130/90. Very pale; feeling sick; headache. Legs as above. Arm reflexes only just present. Tone in arms diminished. Power appears normal. Sensation normal.

1.0 p.m. Feeling ill. Wishes to pass urine but is unable to do so. Headache very marked. Trying to vomit. Arms and legs as before. Given atropine sulphate 2 mg. intravenously without effect.

2.0 p.m. B.P. 150/95. Vomited after eating a piece of chocolate. Condition as before.

3.30 p.m. B.P. 140/90. Tone returning in arms and legs. Arm reflexes now obtainable. Leg reflexes doubtful. Cannot pass urine. Sensation normal.

4.30 p.m. B.P. 145/85. All reflexes now back to pre-injection level. It was found next day that urine had not been passed until 4 a.m. As the bladder never became very distended, it was considered unnecessary to catheterise this patient.

The combined action of prostigmine and acetylcholine in the doses used was thus far greater than the algebraic sum of the action of the two drugs given separately (as the acetylcholine given alone was without effect). It can be concluded that prostigmine given intrathecally markedly potentiated the action of the simultaneously administered acetylcholine. As previously explained, prostigmine given intravenously in the doses used does not have this potentiating effect.

Action of Eserine Sulphate.

This substance was injected intrathecally six times in four cases in doses of 0·25 mg. to 1 mg. The cases used were:

1 cerebral thrombosis.
2 disseminated sclerosis.
1 spinal block.

The results obtained were essentially the same in all cases; one case of disseminated sclerosis and the case of spinal block will be described in detail.

S. W. Case of recovery after cerebral thrombosis. Muscle tone normal; plantar responses extensor. All deep reflexes normal, but power slightly reduced in the arms and legs. Sensation normal.

17.12.37.

10.15 a.m. B.P. 120/90. Lumbar puncture and injection of 0·5 mg. of eserine sulphate. Pulse 70. Grips 70 and 75.

10.45 a.m. No change; feels quite well.

11.0 a.m. Feels quite well, but the legs are "heavy," though the patient moves them at least as well as before the injection. Reflexes unchanged in legs and arms. Pin-prick sensation said to be very sharp in the right leg. Pulse 72; B.P. 130/85.

11.15 a.m. Ankle jerks diminished, but obtained easily. Knee jerks diminished. Arm reflexes not so brisk. Patient now complains of hot sensation in legs and says that the soles feel as though they are being scratched. Pin prick is still sharper than normal. Power in legs appears to be increased.

11.35 a.m. Pulse 64; B.P. 125/85. Now reports tingling sensations in legs instead of warmth. All reflexes enhanced over pre-injection levels. Ankle clonus now appears for the first time.

12.0 p.m. Complains of severe headache; feels sick and attempts to vomit. Cannot bear the contact of the bedclothes as they seem to be hurting the legs. Pin prick not now appreciated so acutely as during the previous hour. Reflexes are as at 11.35 a.m. —*i.e.* still slightly exaggerated and ankle clonus present. Wishes to pass urine but cannot. Injection of 1·5 mg. atropine intravenously had no effect.

12.45 p.m. B.P. 130/80. Headache still very severe. Vomited twice. Reflexes all present and brisk. Tone unaffected. Hand grips both 90 (pre-injection level 70, 75). Complains of cramps in the legs, but no muscle spasm could be seen or felt. Passed urine normally.

2.0 p.m. Trying to sleep, but headache is too severe to allow this. Patient says legs now feel cold and shivery though not cold to the touch. Patient objects to the legs being stroked with the fingers as this is painful.

3.0 p.m. Apart from headache, patient now appears to be in pre-injection state.

Certain features of this experiment require further comment. This patient had made an almost complete recovery following a cerebral thrombosis. Tone and the deep reflexes were practically normal. Following the intrathecal injection of eserine sulphate there were striking subjective sensory disturbances such as warmth in the legs and tingling feelings. Sensory changes were never observed following injection of prostigmine. After a preliminary period of depression of reflexes, beginning half an hour after the injection and lasting for one hour, these returned to a degree greater than before the injection. Ankle clonus, which had not been obtained for three weeks, was now present. This effect lasted for two hours. There was some increase too in muscle power. The usual pallor and vomiting were seen 2½ hours after the injection.

H. M. Case of spinal block due to secondary carcinoma, confirmed by lumbar puncture. Block at level of 5th thoracic segment. There was complete flaccid paralysis of the legs and loss of knee jerks and ankle jerks; extensor plantar responses present. There was complete loss of sensation and of all reflexes up to 5th thoracic segment. No spasms had occurred for three months, though these had been present previously.

17.3.39.

11.0 a.m. Lumbar puncture; intrathecal injection of 0·5 mg. of eserine sulphate.

2.0 p.m. B.P. 150/95. Vomited after eating a piece of chocolate. Condition as before.

3.30 p.m. B.P. 140/90. Tone returning in arms and legs. Arm reflexes now obtainable. Leg reflexes doubtful. Cannot pass urine. Sensation normal.

4.30 p.m. B.P. 145/85. All reflexes now back to pre-injection level. It was found next day that urine had not been passed until 4 a.m. As the bladder never became very distended, it was considered unnecessary to catheterise this patient.

The combined action of prostigmine and acetylcholine in the doses used was thus far greater than the algebraic sum of the action of the two drugs given separately (as the acetylcholine given alone was without effect). It can be concluded that prostigmine given intrathecally markedly potentiated the action of the simultaneously administered acetylcholine. As previously explained, prostigmine given intravenously in the doses used does not have this potentiating effect.

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5 mg. are injected *intravenously* they produce an *increase in muscle tone and reflexes* and muscle twitching which are due to the well-known peripheral effects on skeletal muscle. On the other hand, when very much smaller doses of prostigmine are injected *intrathecally* they produce the exactly opposite effect of *decreasing muscle tone and reflexes,* while no muscle twitching can be observed. It is improbable that the effects of intrathecally injected prostigmine are due to local alterations in the circulation in the spinal cord. The local vasodilation which may occur is unlikely to modify the reflexes adversely. There is no reason for supposing that prostigmine constricts blood-vessels by a direct action, and even if it did, moderate degrees of spinal anæmia have been shown experimentally [Schweitzer and Wright, 1937 *a*] to increase spinal reflexes. A very severe degree of spinal anæmia does ultimately abolish all reflex activity. Such grave anæmia might be expected to interfere with certain forms of sensation at least; no change in sensation was, however, observed in the clinical cases studied. This last finding also demonstrates that the depressant action of prostigmine on spinal reflexes is not due to a peripheral anæsthetic effect. Although the direct action of prostigmine on nerve fibres has not been examined in man, it has been studied in animals. Schweitzer and Wright [1937 c] applied various concentrations of prostigmine, higher than those employed in these experiments, directly to the femoral nerve in cats and found there was no depression of the knee jerk. From these results it can be concluded that intrathecal prostigmine probably does not act on the nerve trunks or on the tracts of the cord. The depression in spinal reflex activity occurs prior to the onset of nausea and vomiting when they are present, and so cannot be attributed to this extraneous factor. In fact, by suitably grading the dose, or in cases with spinal block, depressed spinal reflex activity is produced without any gastro-intestinal symptoms at all. The changes in pulse-rate, blood pressure, and respiration in the long series of cases studied were all of a minor character and bore no relationship to the effects observed on spinal reflexes.

The mode of spread of the symptoms after intrathecal injection of prostigmine is characteristic and is presumably due to progressive involvement of the spinal cord from below upwards. The ankle jerks are depressed before the knee jerks and the leg reflexes before the arm reflexes. Involvement of the face is rare and always occurs last. The way in which the paresis marches from one part to the next fits in quite well with an upward diffusion of prostigmine in the cerebro-spinal fluid and the resulting penetration into and involvement of higher levels of the spinal cord.

In the cases with spinal block, prostigmine inhibits the motor reflex functions below the block only. There are no symptoms above the level of the block and no systemic effects are observed. The common

11.35 a.m. No change seen. Patient says he is quite comfortable, though legs feel as though they are "going to jump."

12.10 p.m. Patient reports no change and no subjective sensations in the legs. Fibrillary contractions are present in all the leg muscles. Crossed adductor reflexes obtained by striking the patellar tendon. General withdrawal of legs results from stimulation of the soles of the feet. No objective changes in sensation.

1.40 p.m. Slight increase of power in legs. Right knee jerks now definitely present.

3.50 p.m. No subjective changes. Power still increased. Tone, particularly in flexors, increased. Right-knee jerk and hamstring jerk further increased and now brisker than in a normal subject. Right-ankle jerk present. Left-knee jerk and hamstring jerk present. Left-ankle jerk not obtained. No further fibrillation of muscles seen.

5.0 p.m. Condition now much as in pre-injection period.

At no time was there any effect on the general condition or on any part of the body above the level of the block.

Both these cases which are reported in detail showed responses which are in marked contrast to those obtained with prostigmine. The remarkable sensory stimulation in the case of *S. W.* has already been commented on. All the cases studied showed after an initial delay a preliminary depression, followed by a slight or a marked increase in tone and reflexes and in power above the pre-injection level. This was especially striking in the case of *H. M.* with spinal block, in whom the results were dramatic; in this patient reflexes which had been absent for months or never elicited previously, made their appearance, following the injection of eserine. As no changes of this kind were observed above the level of the spinal block in the case of *H. M.* the increased motor responses obtained, including the so-called "fibrillary" contractions, can reasonably be attributed to direct stimulation of the spinal cord.

Discussion.

Site and Mode of Action of Prostigmine.

The experiments carried out with prostigmine gave absolutely uniform results and prove conclusively that when this drug is injected intrathecally in man it invariably depresses spinal reflexes and often decreases the strength of voluntary movements. The control experiments show clearly that the changes observed are due to the action of the drug and not to the introduction of some non-specific foreign substance into the theca. The results described cannot be attributed to the passage of prostigmine from the cerebro-spinal fluid into the general circulation. It has been shown that when doses of prostigmine up to

were injected intrathecally in man. Loss of voluntary power was also a frequent occurrence and was often marked. The retention of sensory transmission, together with interference with various forms of spinal transmission to skeletal muscle, suggests that prostigmine may be acting in close proximity to the anterior horn cells.

Mode and Site of Action of Acetylcholine.

Intrathecal injection of acetylcholine in man in doses up to 500 mg. were without effect, on the spinal cord or elsewhere. Furthermore, *intravenously* injected prostigmine failed to potentiate the effects of intrathecally injected acetylcholine. Quite small doses (0·1 mg.) of prostigmine injected intrathecally, together with small doses (10 mg.) of acetylcholine, markedly potentiate the effects of the latter, which then produces depression of the spinal cord resembling that of much larger doses of prostigmine alone. The site of action of acetylcholine may reasonably be supposed to be in the same region as that of prostigmine. This would imply that before intrathecally injected acetylcholine can act it must penetrate through the outer thick layer of spinal white matter to reach the deeper grey matter. Though cerebro-spinal fluid contains little choline-esterase, large concentrations of this enzyme are present in the central nervous system itself [Stedman and Stedman, 1935; Nachmansohn, 1937, 1938]. The grey matter is particularly rich in esterase, which may be supposed to destroy rapidly the acetylcholine slowly penetrating to it, and thus prevent an adequately high concentration of the drug being attained to produce an effect. Furthermore, the relative lipoid insolubility of acetylcholine may hamper its transfer through the substance of the spinal cord. The results of Henderson and Wilson [1936] who noted marked effects in man with acetylcholine may perhaps be due to the fact that they injected the drug directly into the empty lateral ventricles, and so avoided dilution with a large volume of cerebro-spinal fluid.

The results of Schweitzer and Wright [1937] with acetylcholine in anæsthetised cats were in the main identical in character with those produced by prostigmine; they found that prostigmine or eserine potentiated the central depressant action of acetylcholine. Similar results were obtained by Bülbring and Burn [1941] on the knee jerk in dogs, and Torda [1940] on the crossed extensor reflex in toads (using the isolated perfused spinal cord).

All these workers obtained evidence that acetylcholine may have a central stimulating action too. Bülbring and Burn [1941] obtained a discharge from the isolated perfused spinal cord of the dog following injection of acetylcholine, especially after previous administration of eserine. No evidence of such central stimulating action of acetylcholine was obtained in man.

occurrence of nausea and vomiting in cases without spinal block would seem to be due to an action of prostigmine on some supra-spinal level. It may be concluded that intrathecally injected prostigmine in man acts directly on the spinal cord, either on nerve cells or on a synaptic region, *i.e.* on what is now often termed the "neuropile." Schweitzer and Wright [1937 *a*, *b*], using doses of prostigmine of the order of 1 mg. in cats (weighing about 3 kg.), found that intravenous injection depressed spinal reflexes. Appropriate experiments proved that the effects were largely due to a direct depressing action on the spinal cord. The failure of intravenously injected prostigmine to produce similar results in man may be reasonably attributed to the fact that the doses employed in the cats per kilogram body weight were relatively much larger.

Torda [1940], using the isolated perfused spinal cord of toads, found that prostigmine abolished the crossed and ipsilateral extensor reflex and reversed ipsilateral inhibition of the extensor muscle. Perfusion pressures of *50 cm. Hg* were used in these experiments, *i.e.* pressures about 20 times as high as the normal arterial pressure in amphibia. According to Bülbring and Burn [1941] prostigmine in doses of 2 mg. *injected into the arterial cannula carrying blood to the isolated perfused* spinal cord of the dog produced a discharge of nervous impulses as shown by muscular contraction in the legs; these effects were abolished by atropine. In the same preparation prostigmine in doses of 0·5–1 mg. diminished, and 2–5 mg. abolished, the knee jerk. The flexor reflex, however, was usually increased; as this last effect was apparently only observed in the presence of adrenaline in the circulation, its significance is obscure. It is perhaps unwise to assume that results obtained with the isolated perfused spinal cord will necessarily agree with those found in the normal animal. As the flexor reflex in their preparation failed spontaneously unless adrenaline was added to the perfusion circuit, the spinal cord appears to have suffered some alteration in its normal properties in these experimental conditions. One should also be cautious in applying results obtained in lower animals too readily to man.

The results recorded in this paper give no support to the view that prostigmine, in addition to its undoubted central depressant action, may also exert a central stimulating action on *flexor* reflexes. In the case *J.P.* prostigmine abolished all the manifestations of activity of the spinal flexor reflex arc. Flexor posture, flexor spasms, and the plantar so-called "extensor response" all disappeared under the influence of the drug. This last-mentioned reflex is generally regarded as part of the human flexor or withdrawal reflex. In all the patients with pyramidal tract involvement the plantar "extensor response" which was initially present was abolished by prostigmine.

Retention of urine from inability to empty the bladder voluntarily or reflexly was a common finding when the larger doses of prostigmine

preceded by a short period of central depression. As has already been stressed no excitatory manifestations, motor or sensory, have been noted in our very long series of experiments with prostigmine.

It is premature at this stage to speculate as to the cause of the differences thus noted between prostigmine and eserine. Schweitzer, Stedman, and Wright [1939] drew attention to certain physical properties of the anticholine-esterases which may account in part for the variable results obtained. They concluded from their observations that the anticholine-esterases produced their effects on the spinal cord by their specific action of preserving acetylcholine. The quaternary compounds, like prostigmine, give rise in the body to a quaternary base, which is lipoid-insoluble and may thus be limited in its action to the region outside the nerve cell. The tertiary compounds, like eserine sulphate, give rise to the lipoid-insoluble cation and a lipoid-soluble tertiary base which might penetrate the cell membrane. The insoluble derivative of eserine might be conceived to act like prostigmine, while the convulsant action would be due to the lipoid-soluble component. Though this suggestion may help to account for the mixed excitatory-depressant action of eserine, it does not account for the central excitatory action of prostigmine which has been recorded in some species.

Whatever may be the interpretation, the findings in the experiments in man are quite clear cut. Prostigmine, acetylcholine, and eserine injected intrathecally have a direct action on transmission in the spinal cord. Prostigmine depresses transmission through motor arcs, diminishing muscle tone and reflexes (both extensor and flexor) and voluntary power. There is no change in sensation. Acetylcholine potentiated by small intrathecal doses of prostigmine acts similarly. Eserine, after initial motor depression, produces central excitation (increased muscle tone and reflexes), together with enhanced sensory reactions.

Summary.

1. Prostigmine was injected in doses of 0·1–1·5 mg. intrathecally 73 times in 37 patients (with evidence of pyramidal tract involvement) and in 3 subjects with normal central nervous system. In all cases it produced depression of muscle tone and reflexes by a direct action on the spinal cord. The distal part of the cord was involved first and the depression gradually ascended to involve the centres controlling the arms. The face was involved rarely. Changes in blood-pressure, pulse-rate and respiration were small and irregular. Nausea, vomiting, and drowsiness commonly occurred when large doses were employed. Voluntary movement was impaired. Bladder emptying was temporarily abolished. No changes in sensation were ever noted. Observations were made in patients with spinal block. Injection of prostigmine intrathecally below the level of the block produced depression of the

Site and Mode of Action of Eserine.

The evidence in the literature on animal experiments suggests that the action of eserine sulphate on the central nervous system is complex. Schweitzer and Wright [1937] demonstrated in cats under chloralose anæsthesia that eserine injected intravenously increased the knee jerk and produced general convulsions mainly as the result of a central stimulating action on the spinal cord. They quoted a number of observations by previous workers pointing in the same direction. Subsequently Merlis and Lawson [1939] in the dog found that eserine (injected intravenously or intrathecally) increased the flexor reflex. Bülbring and Burn [1941] found that eserine (1·5 mg. in 600 c.c. of blood) caused a spontaneous discharge of impulses from the isolated perfused spinal cord of the dog. Results of a similar nature were obtained by Gellhorn, Darrow, and Yesinick [1939], Hall and Goldstone [1940], Chute, Feldberg, and Smyth [1940], and McKail, Obrador, and Wilson [1941]. There is thus good evidence that eserine may exert a central stimulating action. But there are also a series of observations indicative of a central depressant action of eserine. In some of their experiments on cats Schweitzer and Wright [1937] found that eserine produced an initial depression of the knee jerk, which on occasions was very marked and prolonged. Merlis and Lawson [1941] in the dog found that eserine usually depressed the knee jerk under sodium barbital anæsthesia, and depressed it in 50 per cent. of experiments under chloralose anæsthesia (in the other 50 per cent. stimulation occurred). Bülbring and Burn [1941] found the knee jerk was regularly depressed by eserine (perfused cord, dog). There is also evidence for a mixed excitatory-depressant action on the cerebral cortex, excitation being observed by Miller [1937], Sjöstrand [1937], Miller, Stavraky, and Woonton [1940], Tinel [1937], and Williams and Russell [1941]; depression by McKail, Obrador, and Wilson [1941].

The results recorded in this paper on man give evidence of a direct stimulating action of eserine on the spinal cord. In all the cases studied there was *at some stage* an increase in tone and reflexes, and sometimes in power of voluntary movement. Both flexor and extensor reflexes were enhanced equally. Increase of nystagmus was also observed in one case. The case with spinal block was especially noteworthy, as here eserine caused reflexes to appear in an enhanced form which had been absent for several months. In addition the site of action of eserine in this patient could only have been the spinal cord itself and not higher levels of the central nervous system. There were also striking subjective sensory manifestations (spontaneous pain, burning sensations) and an apparent increase of sensory transmission, though this could not always be established with certainty. But as in some of Schweitzer and Wright's animal experiments the phase of excitation was always

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spinal cord limited strictly to the cord below the level of the block. No general disturbances such as nausea or drowsiness developed. In such cases flexor tone, flexor spasms, and flexor reflexes were abolished at the same rate and to the same degree as extensor reflexes. All the above actions of prostigmine are unaffected by subcutaneous injections of atropine. They contrast markedly with the results of injection of prostigmine intramuscularly or intravenously.

2. Intrathecally injected acetylcholine in doses of 2–500 mg. had no effects on the spinal cord or elsewhere. 10 mg. of acetylcholine injected together with 0·1 mg. of prostigmine produced effects on the spinal cord in every way similar to those of larger doses of prostigmine.

3. Eserine sulphate in doses of 0·25–1 mg. injected intrathecally produces an initial transient depression of spinal reflexes followed by a rapid return to a level exceeding that noted prior to the injection. Experiments in a case of spinal block show that both the initial depression and the secondary excitation are due to a direct action on the spinal cord—the effects being limited to the cord region distal to the block. In this case too the excitatory phase of the response was particularly striking, involving both flexor and extensor reflexes. *Unlike prostigmine*, eserine produces striking sensory changes including facilitation of sensory transmission. Suggestions are made to explain the difference in central action of prostigmine and eserine.

This work was interrupted by the outbreak of the War. I gratefully express my thanks to Professor Francis Fraser in whose department the research was carried out, to Professor Samson Wright for his help in the preparation of the paper, and to the Medical Officer of Health, London County Council, for permission to include the notes on the patients.

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IN VIVO SURVIVAL IN THE HUMAN SUBJECT OF TRANSFUSED ERYTHROCYTES AFTER STORAGE IN VARIOUS PRESERVATIVE SOLUTIONS.

By P. L. MOLLISON and I. M. YOUNG.

A REPORT TO THE MEDICAL RESEARCH COUNCIL FROM THE SOUTH-WEST LONDON BLOOD SUPPLY DEPOT.

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INTRODUCTION.

ALTHOUGH stored blood is now so widely used for transfusion, there still exist considerable differences of opinion as to its efficacy. The value of stored blood, as opposed to that of stored plasma or serum, lies predominantly in the ability of its erythrocytes to perform their biological function in the recipient's circulation after transfusion, and the other cells and substances in stored blood are generally agreed to play a relatively unimportant rôle. Although this proposition is generally accepted, only a few workers have attempted to measure the *in vivo* survival of the transfused erythrocytes of stored blood. The majority of the clinical studies have been devoted to demonstrating the safety rather than the efficacy of stored blood [DeGowin and Hardin, 1940; Stewart, 1940]. Great attention has been paid, however, to the changes occurring in the erythrocytes *in vitro* during storage, and many workers have assumed [Denstedt *et al.*, 1940] or endeavoured to demonstrate [Bushby *et al.*, 1940] that these changes are correlated with the *in vivo* survival of the same erythrocytes after transfusion. The changes most commonly studied are the degree of spontaneous hæmolysis and alterations in the osmotic fragility of the erythrocytes.

In a previous paper [Mollison and Young, 1941] it was pointed out that in fact these two commonly measured *in vitro* properties are not related in any constant way to *in vivo* survival, and that therefore conclusions based upon such tests might be entirely fallacious. Two examples will emphasise this point. The erythrocytes of blood stored with the Rous-Turner preservative solution rapidly become very fragile to hypotonic saline solutions. Nevertheless, when transfused after 18 to 21 days' storage, these erythrocytes are found to survive in the recipient's blood-stream almost as well as those of fresh blood. Conversely, the erythrocytes of blood stored with sucrose become

TABLE I.—COMPOSITION OF MAIN SOLUTIONS USED AND AVERAGE PERCENTAGE SURVIVAL *IN VIVO* OF GROUPS OF SAMPLES AFTER STORAGE IN THESE SOLUTIONS.

Preservative.		Length of storage in days.	No. of cases.	Per cent. survival *in vivo*.			
				24 hours.	1 week.	3 weeks.	2 months
A. SOLUTIONS CONTAINING NO CARBO-HYDRATE.							
I. *Sodium citrate.*	c.c.						
Sodium citrate 3 per cent.	*100*	5–9	3	72	68	51	27
Blood	*440*	11–17	3	25	11	..	..
II. "*I.H.T.*"							
Sodium chloride 0·7 per cent.	*270*	8–10	4	78	52	42	20
Sodium citrate 0·52 per cent.							
Potassium chloride 0·02 per cent.							
Mag. sulphate 0·004 per cent.							
Blood	*270*						
III. *Defibrinated blood* (Harrison & Picken).							
Glass beads	*30*	6–9	4	89	76	63	20
Blood	*450*						
IV. *Heparinised blood.*							
"Liquemin" 15,000 ACU or B.D.H. Heparin 2000 units in saline	*10*	6–10	4	76	61	40	6
Blood	*450*						
V. *Excess sodium citrate.*							
Sodium citrate 3 per cent.	*700*	9	2	40	22	10	..
Blood	*300*						
B. SOLUTIONS CONTAINING CARBO-HYDRATE.							
VI. *Citrate-glucose* (M.R.C.)		0–4	15	104	101	86	49
Sodium citrate 3 per cent.	*100*	11–15	4	88	77	60	27
Glucose 30 per cent.	*10*	18–19	3	84	73	53	13
Blood	*430*	23–29	2	52	35	20	..
VII. *Citrate-glucose.*							
Sodium citrate 3 per cent.	*100*	11–14	2	89	70	60	24
Glucose 30 per cent.	*40*	16–21	4	77	65	48	20
Blood	*400*						
VIII. *Citrate-glucose.*							
Sodium citrate 3 per cent.	*100*	20	2	76	61	50	17
Glucose 5·4 per cent.	*100*						
Blood	*340*						
IX. *Citrate-glucose.*							
Sodium citrate 5 per cent.	*40*	18–20	3	88	82	69	19
Glucose 5·4 per cent.	*180*						
Blood	*320*						
X. *Citrate-glucose* (Rous-Turner, 1916).							
Sodium citrate 3·8 per cent.	*200*	17–21	3	100	93	74	37
Glucose 5·4 per cent.	*500*	24–28	4	83	78	50	12
Blood	*300*	34–35	2	54	31	..	..

more resistant than normal to hypotonic saline and undergo little hæmolysis. Nevertheless, when transfused after 15 days' storage, they are almost completely eliminated from the recipient's circulation within 2 to 3 days.

In view of these observations it seemed desirable to measure the *in vivo* survival of the transfused erythrocytes of blood after storage in various preservative solutions, and to discover which of the many recommended solutions were really effective in preserving the biological value of blood during storage. At the same time, the commonly accepted *in vitro* tests were applied to all samples before transfusion, and additional evidence was obtained that the results of these tests are often fallacious guides to the *in vivo* survival of erythrocytes from the same sample.

Methods.

Anticoagulant and Preservative Solutions used.

The compositions of the main solutions tested are given in Table I. (A few solutions containing varying amounts of both sucrose and glucose were also tried and are referred to below.) Solutions I–IV, VI, X, XII, and XIII were all tried because they are in use at present or because their use has been advocated. It became clear at an early stage of the experiments that the Rous-Turner solution had far the most favourable effect upon subsequent *in vivo* survival. Solutions nos. V, VII–IX, and XI were tried in an effort to discover which factor or factors are responsible for this good effect. Thus, no. V reproduces the dilution of the Rous-Turner solution but lacks glucose. No. XI is similar to no. V, but contains the same small final concentration of glucose as the standard (M.R.C.) citrate-glucose solution (no. VI). Three other citrate-glucose mixtures (VII–IX) that were tried contained amounts of glucose intermediate between that in the standard citrate-glucose solution and the Rous-Turner solution.

Principle of Method of following in vivo Survival.

The fate of the transfused erythrocytes was investigated by a modification [Mollison and Young, 1940] of the method of Ashby [1919]. Patients of group A (or B) are transfused with blood of group O. After transfusion, the recipient's blood contains two types of cell, O and A. If samples are then taken, all but a few thousand of the A cells can be agglutinated with anti-A serum and the free (group O) cells of the donor counted. The small fraction of A cells that are not agglutinated are estimated before transfusion, and this number is deducted from the total "inagglutinable" count to give the actual number of donor cells surviving. Sera of high titre are used and all counts are performed in duplicate.

6·6 million per c.mm., and on the average 475 c.c. of cell suspension were transfused.

The use of concentrated cell suspensions [Castellanos, 1937; MacQuaide and Mollison, 1940] has many advantages. Despite the great variations between the proportions of blood and preservative solutions in the different mixtures, suspensions of approximately similar red-cell concentration can be given in most cases. Thus a similar volume of suspension can be used in the majority of transfusions, and blood-volume changes in the recipient tend to be standardised. At the same time the total amount of fluid introduced into the recipient's circulation is reduced, and this is of value in reducing blood-volume changes.

Since the donor was usually group O and the recipient group A, the use of concentrated cell suspensions had the additional advantage of reducing to a minimum the volume of "incompatible" plasma transfused. The introduction of a large volume of O plasma into the circulation of patients of other groups is usually regarded as undesirable, particularly when the titre of the agglutinins is high.

Clinical Material.

The recipients were all patients suffering from secondary anæmia. Cases in which hæmolytic anæmia or intra-current hæmorrhage was suspected have not been included. Many of the patients were women who had been recently delivered and who were found to be suffering from a moderate hypochromic anæmia.

Sampling of the Recipient.

A venous sample was taken from the recipient immediately before the transfusion was started. The transfusion was usually completed in 60–120 minutes. Five minutes after the end of the transfusion a further sample was collected, and a third sample was taken at some point 4–8 hours from the beginning of the transfusion. The next sample was usually collected 24 hours after transfusion and thereafter samples were obtained at convenient intervals.

When cell suspensions prepared from blood stored for only a few days were transfused, it was found that the concentration of donor cells was usually greater in the 4–8-hour sample than in that taken immediately after transfusion (see fig. 1, curve A). This suggests that the blood-volume is increased at the end of transfusion and that the excess volume is at least partly removed during the following few hours. Samples taken at the end of 24 or 48 hours in such cases usually showed a further increase in the concentration of donor cells (see fig. 1), suggesting that readjustment of blood-volume is not as a rule completed within a few hours of transfusion. When older blood is administered,

TABLE I—*continued.*

Preservative.		Length of storage in days.	No. of cases.	Per cent. survival *in vivo.*			
				24 hours.	1 week.	3 weeks.	2 months.
B. SOLUTIONS CONTAINING CARBOHYDRATE—*continued.*							
XI. *Citrate-glucose.*							
Sodium citrate 3 per cent.	*700*	16–19	2	53	40	29	12
Glucose 30 per cent.	*20*						
Blood	*280*						
XII. *Dextrin* (Maizels, 1940).							
Sodium citrate 3 per cent.	*100*	9	1	82	76	62	30
Dextrin 53 per cent.	*30*	12–13	2	86	57	31	..
Blood	*410*						
XIII. *Sucrose* (Wilbrandt, 1940).							
Sodium citrate 10 per cent.	*63*	7–9	2	85	76	66	34
Sucrose 10·3 per cent.	*375*	12–16	6	55	33	25	8
Blood	*410*						

In a few cases, in which the recipient belonged to group O, survival was estimated after differential agglutination using anti-M or N sera and making use of differences in M and N type between donor and recipient [Landsteiner, Levine, and Janes, 1928; Wiener, 1934].

Use of Concentrated Erythrocyte Suspensions for Transfusion.

Blood was taken from supposedly healthy donors into the various solutions and stored at 3° to 7° C. until required for transfusion. Bottles with a capacity of 540 c.c. were usually used, but in the cases of solutions V, VII, and XI three-litre bottles were used because of the large volume of these solutions.

On the day of transfusion two or more bottles of identical age, and containing blood stored with the same anti-coagulant solution, were carefully removed from the refrigerator, to avoid mixing of cells and plasma. The bulk of the supernatant plasma was then siphoned off and the red cells from the bottles concerned were pooled and well mixed. The bulk of this concentrated erythrocyte suspension was used for transfusion, but a small sample was retained for counting and testing. The concentration of erythrocytes in these suspensions was found to vary between 4·5 and 8·4 million R.B.C. per c.mm. The lower figures were found when blood that had only been stored for very short periods was used, and with the Rous-Turner solution in which the cells do not become tightly packed. The higher figures were found with the oldest bottles of blood and particularly with those stored with sucrose, in which preservative solution the erythrocytes become very tightly packed. The average red-cell concentration was

changes are similar in the majority of cases, and this compromise therefore enables quite accurate comparisons to be made.

A further point must be considered. When old stored blood is transfused, cell destruction is found to be occurring during the few hours after transfusion, and it is probable that it has been taking place during the transfusion. If this destruction during transfusion is appreciable, there will be a further error in taking as 100 per cent. the concentration of donor cells found immediately after transfusion. This point can be investigated by calculating the concentration of donor cells to be expected in the recipient's blood-stream after the transfusion of a measured amount of blood and comparing this theoretical figure with the one actually found. The calculation is made from the formula:

$$\frac{\text{Concentration of erythrocytes in donor blood}}{\text{Concentration of donor erythrocytes in recipient's blood-stream}} = \frac{\text{Blood-volume of recipient (after transfusion)}}{\text{Volume of donor blood}}.$$

The recipient's blood-volume was calculated from known height and weight using the table of Gibson and Evans [1937]. This calculation was made for every transfusion, and it was found that only in the case of a few of the samples of stored blood which later underwent rapid destruction was the concentration of donor cells appreciably lower than expected in the sample taken immediately after transfusion. The mechanism of this early phase of destruction is discussed below.

In making the above calculations certain liberties had to be taken. It is well recognised that the blood-volume is increased in pregnant women [Thomson *et al.*, 1938], and if this had not been taken into consideration the concentration of donor cells found in the recipient's circulation, when pregnant women were transfused, would have been lower than expected. Conversely, in the majority of the non-pregnant cases the concentration of donor cells was usually greater than expected. This is almost certainly due to the fact that the blood-volume is lower than normal in anæmia [Gibson, 1939]. If more extensive data had been available of the blood-volume in anæmia and in pregnancy, it would probably have been better to have used these corrected blood-volume figures and to have calculated the expected concentration of donor cells from them. Then all counts of donor cells could have been expressed as percentages of this expected figure.

Without an accurate estimation of the blood-volume in each case, it seems better to refer to the concentration of donor cells in the first sample as 100 per cent. survival, and to express all subsequent counts as percentages of this figure, even though this involves expressing some figures as 104 per cent. survival, for instance.

however, the concentration of donor cells is usually lower in the sample taken 4–8 hours after transfusion than in that taken immediately after transfusion, and is lower still at 24 hours (see fig. 1, curves B, C, and D).

These observations are of importance in considering the method of calculating the percentage of donor cells that survive. After a fresh blood transfusion the most reliable method would seem to be to take

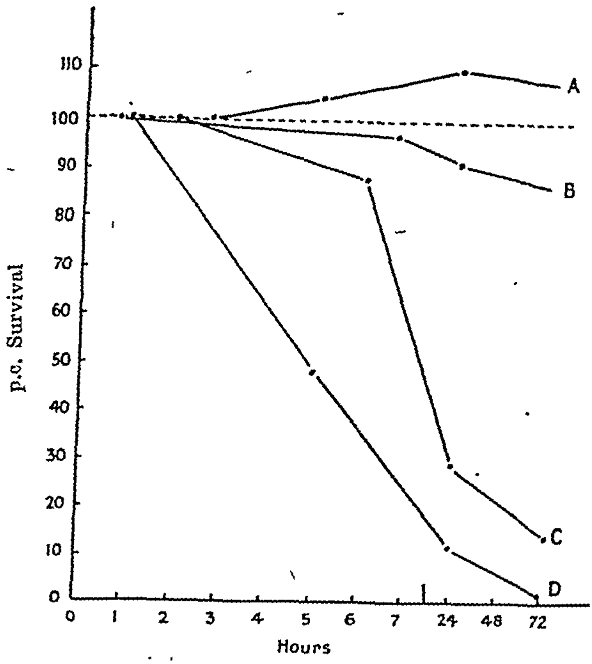

FIG. 1.—Changes in concentration of donor erythrocytes (stored in different solutions) during the 24 hours following transfusion.

A = 4 days old citrate glucose (VI).
B = 19 days old citrate glucose (VI).
C = 16 days old citrate sucrose (XIII).
D = 17 days old citrate (I).

a sample from the recipient 48 hours after the transfusion and then, taking the concentration of donor cells in this sample as 100 per cent., to express subsequent counts as percentages of this figure. In this way, changes due to alterations in blood-volume would be minimised. With stored blood, however, this method is clearly inapplicable, because in many cases the concentration of donor cells will be lower in a sample taken at 48 hours than in a sample taken immediately after transfusion. A compromise may be effected by taking as 100 per cent. the concentration of donor cells found in a sample obtained immediately after transfusion. Provided that approximately the same volume of suspension has been transfused, the results indicate that blood-volume

changes are similar in the majority of cases, and this compromise therefore enables quite accurate comparisons to be made.

A further point must be considered. When old stored blood is transfused, cell destruction is found to be occurring during the few hours after transfusion, and it is probable that it has been taking place during the transfusion. If this destruction during transfusion is appreciable, there will be a further error in taking as 100 per cent. the concentration of donor cells found immediately after transfusion. This point can be investigated by calculating the concentration of donor cells to be expected in the recipient's blood-stream after the transfusion of a measured amount of blood and comparing this theoretical figure with the one actually found. The calculation is made from the formula:

$$\frac{\text{Concentration of erythrocytes in donor blood}}{\text{Concentration of donor erythrocytes in recipient's blood-stream}} = \frac{\text{Blood-volume of recipient (after transfusion)}}{\text{Volume of donor blood}}.$$

The recipient's blood-volume was calculated from known height and weight using the table of Gibson and Evans [1937]. This calculation was made for every transfusion, and it was found that only in the case of a few of the samples of stored blood which later underwent rapid destruction was the concentration of donor cells appreciably lower than expected in the sample taken immediately after transfusion. The mechanism of this early phase of destruction is discussed below.

In making the above calculations certain liberties had to be taken. It is well recognised that the blood-volume is increased in pregnant women [Thomson *et al.*, 1938], and if this had not been taken into consideration the concentration of donor cells found in the recipient's circulation, when pregnant women were transfused, would have been lower than expected. Conversely, in the majority of the non-pregnant cases the concentration of donor cells was usually greater than expected. This is almost certainly due to the fact that the blood-volume is lower than normal in anæmia [Gibson, 1939]. If more extensive data had been available of the blood-volume in anæmia and in pregnancy, it would probably have been better to have used these corrected blood-volume figures and to have calculated the expected concentration of donor cells from them. Then all counts of donor cells could have been expressed as percentages of this expected figure.

Without an accurate estimation of the blood-volume in each case, it seems better to refer to the concentration of donor cells in the first sample as 100 per cent. survival, and to express all subsequent counts as percentages of this figure, even though this involves expressing some figures as 104 per cent. survival, for instance.

The hæmoglobin content and hæmatocrit values of venous samples obtained during the first 24 hours after transfusion were measured. Subsequently, hæmoglobin estimations were made in some cases on capillary samples. The results, as might have been anticipated, were very irregular, since they were affected by so many factors, such as blood-volume changes, the amount of destruction of donor cells, the response of the recipient to the administration of iron, etc. Although in general the most prolonged and substantial increases in hæmoglobin and hæmatocrit values were found after the transfusion of samples that survived best *in vivo*, it would certainly not have been possible to have drawn many reliable conclusions as to the survival of the donor cells from these data, and these results are not referred to elsewhere in this paper.

The bilirubin content of the serum before transfusion, immediately after transfusion, and 4–8 hours after the beginning of the transfusion, was estimated in the majority of instances by a quantitative Van den Bergh method. The serum was also examined by naked eye for evidence of intravascular hæmolysis.

In vitro Tests.

As mentioned above, a sample was taken from each concentrated suspension of stored erythrocytes used for transfusion and kept for testing. Additional material was obtained by taking blood into all the various solutions and keeping samples for the performance of regular tests at 5-day intervals during a period of 28 days. Blood from 3–4 donors was used in the case of each solution. Immediately after bleeding, the total amount was divided into a number of small (1 oz.) bottles so that a previously undisturbed sample was available for the performance of the *in vitro* tests on a given day.

The results obtained with samples that were also used for transfusion and with those kept especially for *in vitro* testing showed no consistent differences and are considered together.

In all cases the tests were performed upon a sample of blood whose hæmoglobin content had been adjusted to 70 per cent. [Haldane]. This standardisation was particularly desirable in determining the resistance of the erythrocytes to shaking because the concentration of red cells clearly affects the result considerably. Also, in determining osmotic fragility a small error is introduced by adding whole blood and not erythrocytes only to the various saline solutions, so that in fact the osmotic pressure exerted by the erythrocyte is being balanced against that of a small amount of plasma and diluent solution as well as a large amount of saline solution. It is therefore desirable to standardise the amount of plasma added in all the tests.

The adjustment of cell suspensions to a hæmoglobin content of

70 per cent. was effected by adding supernatant plasma obtained from another portion of the sample. In performing tests upon fresh blood mixtures, some of the supernatant plasma was removed after centrifugation if the hæmoglobin content was below 70 per cent. initially.

(1) *Spontaneous Hæmolysis.*—The degree of spontaneous hæmolysis is conveniently expressed as

$$\frac{\text{The amount of free hæmoglobin in the plasma} \times 100 \text{ p.c.}}{\text{Total hæmoglobin content of the sample}}.$$

The amount of free hæmoglobin in the plasma was determined after centrifuging a well-mixed sample of cell suspension. The colour of the supernatant plasma was then compared with different standards. The standards were prepared by taking a sample of blood of known hæmoglobin content and then making convenient dilutions (0·1 to 5·0 per cent. Hb—Haldane) with fresh serum as diluent. The total amount of free hæmoglobin in the sample was then calculated from the hæmatocrit estimation by multiplying the percentage of plasma in the sample by the concentration of free hæmoglobin. This amount of free hæmoglobin was then expressed as a percentage of the total hæmoglobin content of the sample. A similar method of estimating spontaneous hæmolysis was suggested by Maizels and Whittaker [1939].

It may be well to emphasise that the amount of spontaneous hæmolysis cannot be estimated by noting the degree to which hæmoglobin diffuses upwards from the undisturbed erythrocytes in a vessel containing stored blood. This has often been pointed out before, but is still not always appreciated [see Crosbie and Scarborough, 1942, for instance]. Amongst other factors, the mechanical barrier of the leucocyte layer prevents free diffusion of liberated hæmoglobin and a clear supernatant plasma is therefore no guarantee that the underlying erythrocytes are intact.

(2) *Osmotic Fragility.*—The method of Dacie and Vaughan [1938] was used with minor modifications. Instead of adding one drop of blood to each c.c. of saline, a measured amount (0·04 c.c.) was added. Further, for convenience, the fragilities were determined at room temperature after it had been established that the results differed by only 0·04 per cent. NaCl from those obtained at 4° C. In the majority of cases only two readings were noted, *i.e.* the concentration of sodium chloride in which 50 per cent. of the corpuscles lysed (median corpuscular fragility or M.C.F. of Dacie and Vaughan), and the concentration in which a trace of hæmolysis occurred.

(3) *Effect on Osmotic Fragility of Preliminary Treatment of Erythrocytes with Fresh Plasma.*—A sample of the blood to be tested was spun for two minutes at 2000 r.p.m. After removal of the supernatant plasma, 1 c.c. of cells was taken and mixed with 1 c.c. of fresh, compatible, heparinised plasma, or in some cases with 1 c.c. 0·86 per cent.

NaCl. The mixtures were allowed to stand at room temperature for varying periods and osmotic fragility was estimated before and afterwards.

It should be pointed out that this simple treatment does not provide an ideal opportunity for the diffusion of substances from stored erythrocytes since the fresh plasma is not in excess, as it would be *in vivo* if the cells were used for transfusion.

(4) *Mechanical Fragility.*—5 c.c. of blood, adjusted to a hæmoglobin content of 70 per cent., was placed in a 1-oz. flat bottle with 10 c.c. glass beads of diameter approximately 0·4 cm. The bottle was then shaken for 30 minutes in a mechanical shaker. During this period the bottle was placed horizontally upon its narrow side. The contents of the bottle (without the beads) were then centrifuged and the hæmoglobin content of the supernatant fluid was then estimated.

The figures for mechanical fragility in fig. 10 simply express the hæmoglobin content of the plasma of a sample of standard hæmoglobin content after shaking and centrifuging.

(5) *Mean Corpuscular Volume (M.C.V.).*—M.C.V. was determined from red-cell counts and hæmatocrit estimations.

(6) *pH Changes.*—pH changes of whole stored blood were determined with the glass electrode. These determinations were kindly carried out for us by Dr. Hewitt.

Results.

In vivo Tests.

The majority of the results of the *in vivo* survival tests are presented in Table I. and some of the results are illustrated graphically in figs. 2–7. In order to facilitate comparison, the figures for small groups of cases in which blood of similar age was used has been averaged. In fact, the survival of blood stored in a given solution tended to be very consistent, particularly in the solutions containing glucose, so that in each solution survival bore an approximately inverse relationship to previous length of storage (see figs. 2–7). Only one gross exception to this rule was found (see fig. 7). Because of this consistency it seems permissible to draw conclusions from small groups of cases.

In examining each group of results the survival figures for the cases transfused with blood stored for 0–4 days with citrate-glucose (VI) may be taken as normal survival. In this group no significant difference was found between the cases receiving fresh blood and those receiving 4-day-old blood. It will be noted that the percentage figures are slightly higher than expected from the assumption that transfused fresh blood is steadily eliminated over a period of 80–120 days after transfusion [*e.g.* Wiener, 1934]. This is due to the method of expressing results (see above). 49 per cent. survival, for instance, means that the number

of surviving erythrocytes per cu. mm. is 49 per cent. of that found immediately after transfusion. In this group the concentration of donor cells found at 48 hours was 108 per cent. of that found immediately after transfusion. If this 48-hour figure is instead taken as 100 per cent., then the figure at 2 months (60 days) becomes 45 per cent. Then if 55 per cent. of the cells are eliminated in 60 days, 100 per cent. will be eliminated in $\frac{60 \times 100}{55} = 109$ days, a figure for total survival that agrees well with that of Wiener.

Solutions I–V. (Solutions containing no Carbohydrate.)—The results for small groups of samples of comparable age are set out in

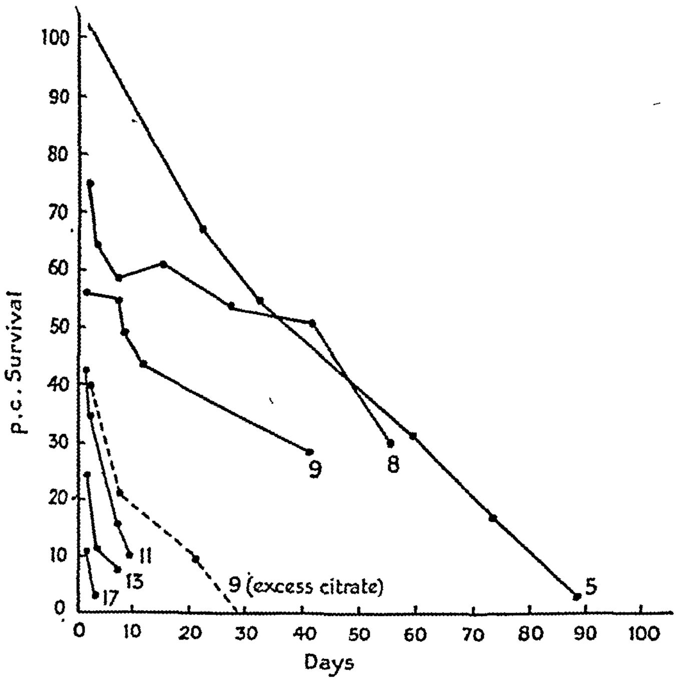

FIG. 2.—Sodium citrate (I).

Figs. 2–7 illustrate graphically the percentage survival *in vivo* of samples stored for different periods. In fig. 4 the curves for citrate glucose VI are averages (taken from Table I.). In the case of the other preservative solution the curves illustrate the survival in single cases. The figures against the curves denote the length of previous storage (in days).

Table I. On the average, blood stored for from 5 to 10 days survives far less well than fresh blood, for although samples transfused after 6 days' storage or less often survive almost as well as fresh blood, the average is brought down by the older samples. There is a suggestion that the survival of stored defibrinated blood is better than that of stored citrated blood (compare figs. 2 and 3). The erythrocytes of

blood stored with sodium citrate for 11–17 days are rapidly eliminated from the circulation, only 25 per cent. remaining at 24 hours. The survival of blood stored with excess sodium citrate (V) is even worse than that of blood stored with the standard amount (I).

Solutions XII and XIII. (Dextrin and Sucrose.)—The slight improvement in *in vivo* survival produced by adding sucrose to citrated

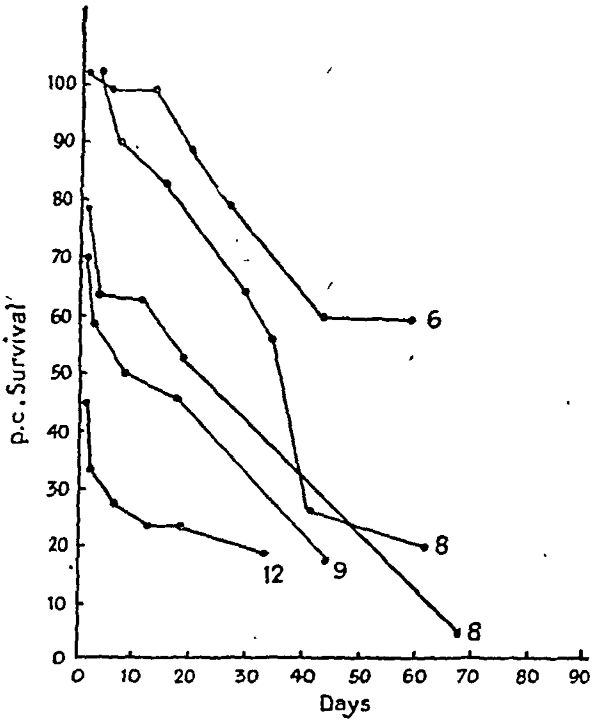

Fig. 3.—Defibrinated blood (III).

blood during storage is shown by comparing the 12–16-day-old group of the former (XIII) with the 11–17-day-old group of the latter (I). It must be pointed out, however, that in one case in which blood stored for 14 days with sucrose was transfused, very good survival was found (see fig. 7). Survival in 4 other cases receiving 14–16-day-old blood was very poor. As mentioned above, this was the only occasion in this entire series of experiments in which such a gross inconsistency was found.

An interesting difference was noted between blood stored with the citrate sucrose and plain citrate mixtures with regard to the rate of elimination of "out-of-date" blood. A typical example is given in fig. 1, where it is seen that although the survival of the two comparable samples (C and D) is similar at 24 hours, the initial destruction of the

citrate sample in the first few hours is far greater. Whereas the erythrocytes of the sucrose sample are eliminated at a more or less uniform rate, a considerable percentage (50 per cent.) of those of the citrate sample are eliminated in the first five hours and the

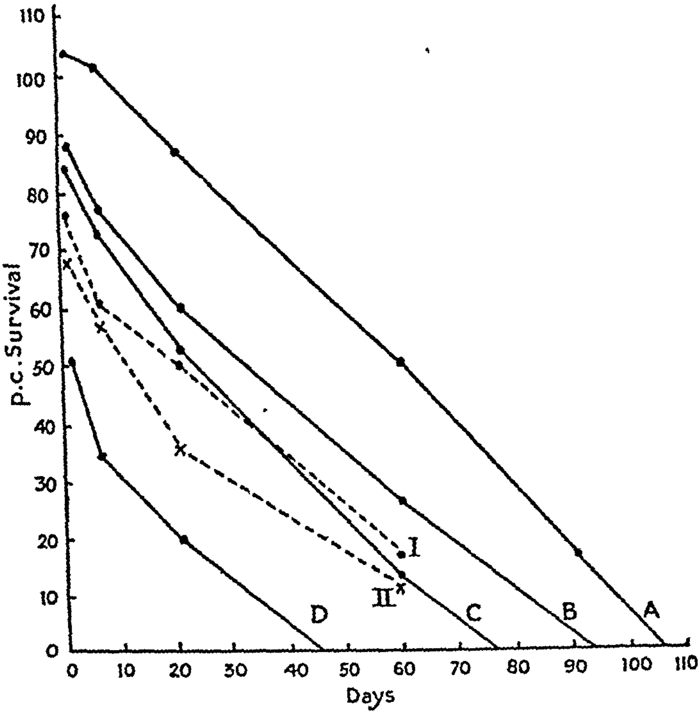

FIG. 4.—Citrate glucose.

A = 0–4 days.
B = 11–15 days.
C = 17–21 days.
D = 23–29 days.
I = citrate glucose (VIII) 20 days.
II = citrate + final 4 per cent. sucrose 20 days.

remainder are then more slowly destroyed. The significance of this difference is not understood, but it was observed in several cases.

The addition of dextrin (XII) to citrated stored blood also appears to prolong survival slightly, although 50 per cent. of the erythrocytes of blood stored for 12–13 days with this preservative are found to be eliminated within one week of transfusion (see fig. 6).

Solutions VI–XI. (*Solutions containing Glucose.*)—When the survival of blood stored with glucose is examined, a great improvement is detectable. Thus in the case of solution VI, for instance, an obvious diminution in survival is not apparent until blood stored for 11–15 days is used (see fig. 4), and even when 18–19-day-old blood is used, 73 per

cent. of the donor cells are found to be surviving in the recipient's blood-stream one week after transfusion. The latter figure may be contrasted with that for the group transfused with blood stored for 11–17 days in sodium citrate only, in which only 11 per cent. of the transfused cells were found to be surviving one week after transfusion.

The survival of blood stored with the Rous-Turner solution (X) (see fig. 5) is considerably better even than that of the standard citrate-glucose solution (VI). Thus, the average survival in a group of 3 cases receiving blood stored for 17–21 days with this solution is not greatly different from that of fresh blood (Table I.). Even when blood stored with this solution for 28 days is used, there is no wholesale rapid destruction of the transfused cells and approximately one-third are still present one month after transfusion.

The survival of blood stored with other citrate-glucose mixtures (VII, VIII, IX) does not seem to be much different from that of blood stored with the standard citrate-glucose solution if the groups of similar age are compared. The survival of the group stored with solution IX, however, does seem to be slightly better than that of the standard citrate-glucose mixture (VI), the difference being most suggestive when the figures found 3 weeks after transfusion are compared (69 per cent. survival compared with 53 per cent. for the standard citrate-glucose in small groups of similar age).

Blood stored with the citrate-glucose solution, which contains a large relative volume of sodium citrate (XI), survives less well than blood stored with the other citrate-glucose solutions.

Additional Preservative Solutions.—In a few cases, combinations of sucrose and glucose were tested for their effect upon preservation. The addition of glucose in a final concentration of 0·6 per cent. to the citrate-sucrose preservative (XIII) or of sucrose (final concentration 4·3 per cent.) to the standard citrate-glucose solution (VI) were both tried. In each case the survival of the blood was not significantly different from that stored with glucose alone, although the *in vitro* properties of the blood were considerably altered (see below).

The addition of sucrose (final concentration of 4·3 per cent.) to the Rous-Turner solution also affects the results of *in vitro* tests made upon the blood with which it is stored; the effect upon the subsequent *in vivo* survival was only studied in one case and the small difference observed cannot be considered significant (see fig. 5).

In case the greater concentration of sodium citrate in the Rous-Turner solution (as used in these experiments) had any effect upon the *in vivo* survival, one case was transfused with blood stored for 21 days in solution VI, modified to contain 4 per cent. instead of 3 per cent. sodium citrate. Survival was very similar to that of blood stored for a similar period with the unmodified solution VI. DeGowin, Harris, and Plass [1940] have pointed out that Rous and Turner used

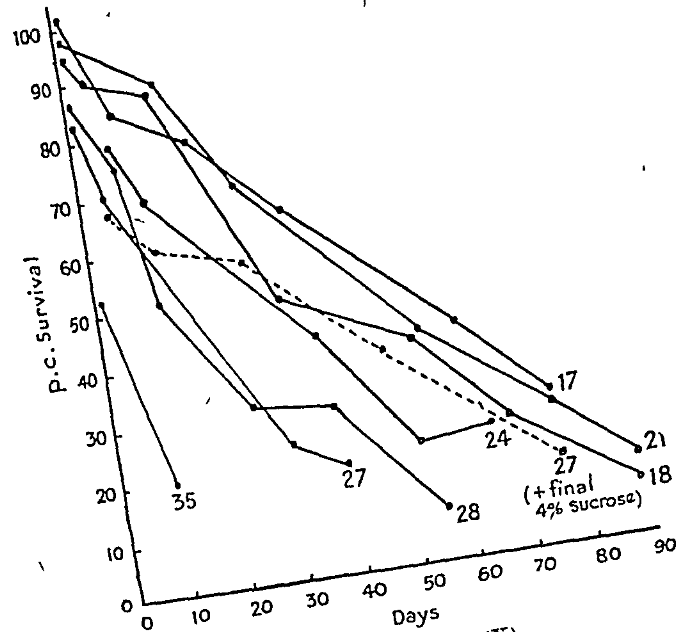

FIG. 5.—Rous-Turner (X).

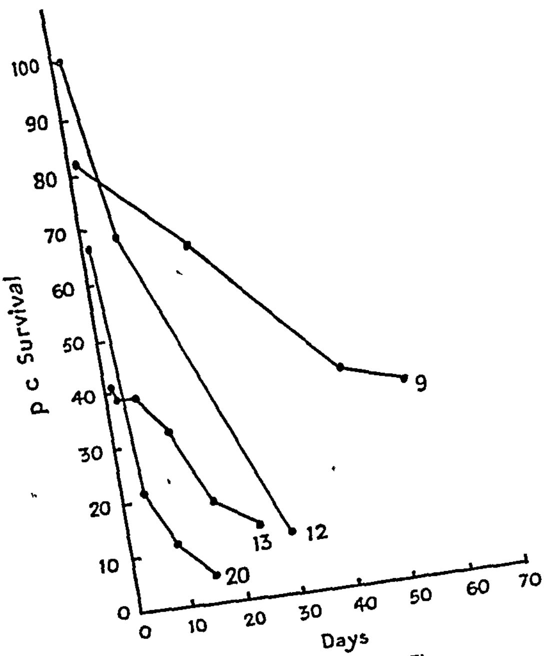

FIG. 6.—Dextrin (XII).

sodium citrate with $5\frac{1}{2}$ molecules of water of crystallisation, whereas in the present experiments citrate with 2 molecules of water was used. The solution used in the present experiments therefore contains a higher concentration of sodium citrate than the original Rous-Turner solution.

The Effect of Preventing Sedimentation.—Fåhraeus [1939] has claimed that when erythrocytes lose contact with plasma, lysolecithin is formed

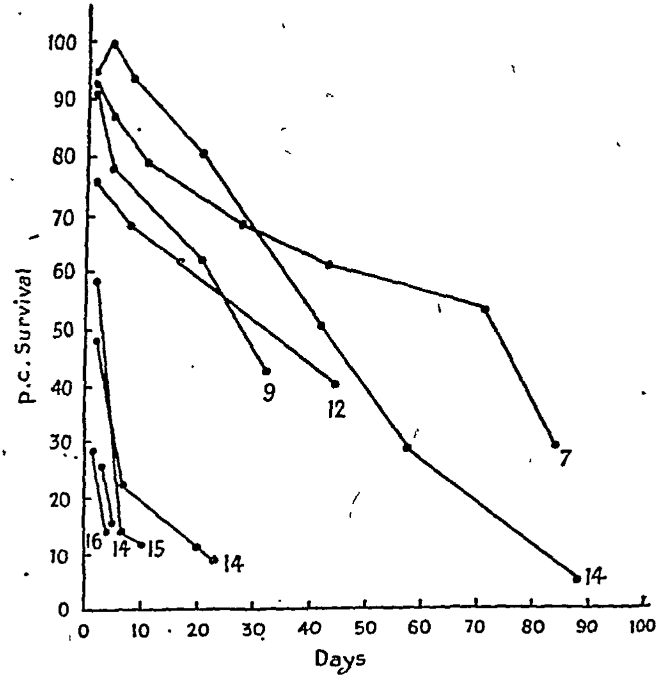

FIG. 7.—Sucrose (XIII).

and the erythrocytes become spherocytic, and are then, according to him, half-way to hæmolysis. The development of lysolecithin can be prevented by keeping cells and plasma in contact—by agitating the blood, for instance. A simple experiment was devised to see whether, if stored blood were prevented from sedimenting, the survival of the cells after a given time would be improved.

Blood was taken into the standard citrate-glucose mixture and the bottles were placed in a refrigerator in the ordinary way. During the first 24 hours the bottles were inverted and mixed several times; on each of the next four days they were mixed several times; on each of the next four days they were mixed and inverted every 12 hours, and thereafter once a day. This simple treatment did not completely prevent sedimentation, but it ensured a very much greater degree of contact between cells and plasma than usually occurs during storage.

At the end of 19 days, 2 bottles of blood treated in this way were given to each of 3 patients and the *in vivo* survival of the erythrocytes was followed. Survival was found to be less good, if anything, than that of blood stored for the same period in the same solution but allowed to sediment completely.

Serum Bilirubin Changes.—The bilirubin content of serum samples obtained from the recipient at intervals after the transfusion of blood stored in different solutions is set out in Table II.

TABLE II.—CHANGES IN SERUM BILIRUBIN CONCENTRATION FOLLOWING TRANSFUSIONS OF STORED BLOOD.

Preservative.	Time of storage (days).	No. of cases.	Serum Bilirubin, mgm. per cent.			
			Before.	0–2 hours after.	4–6 hours.	24 hours.
A. Those containing no glucose, *i.e.* I–V, XII, and XIII.	5–10 11–17	19 11	0·1 0·1	0·5 0·7	1·2 2·4	0·3 0·5
B. Citrate-glucose mixtures, except Rous-Turner.	0–5 11–15 17–21	15 6 16	0·0 0·0 0·1	0·3 0·2 0·7	0·5 0·7 1·3	0·0 0·0
C. Rous-Turner (X) . .	17–21 24–28	4 4	0·0 0·1	0·3 0·3	0·9 1·1	0·3 0·3

To show the relationship between the degree of *in vivo* destruction of donor blood and the increase in the bilirubin concentration in the recipient's serum, the cases have been divided into three groups according to the results of the *in vivo* survival experiments.

Samples were taken before, immediately after, 4–6 hours after (4–8 hours from the beginning), and 24 hours after transfusion. The time 4–6 hours was chosen because it has been shown that the rise in serum bilirubin after the transfusion of stored blood is maximal at this time [Vaughan, 1942].

It is clear from the table that after the transfusion of stored blood of a given age the rise in bilirubin is greater 4–6 hours after transfusion than it is immediately after transfusion, and that by the end of 24 hours the level has practically returned to that prevailing before the transfusion. The low initial figures are to be explained by the fact that almost all the recipients were suffering from a secondary anæmia.

The greatest increase in serum bilirubin was found after the transfusion of blood stored without glucose, that is to say in cases in which the greatest amount of destruction of donor blood occurs, and the

sodium citrate with $5\frac{1}{2}$ molecules of water of crystallisation, whereas in the present experiments citrate with 2 molecules of water was used. The solution used in the present experiments therefore contains a higher concentration of sodium citrate than the original Rous-Turner solution.

The Effect of Preventing Sedimentation.—Fåhraeus [1939] has claimed that when erythrocytes lose contact with plasma, lysolecithin is formed

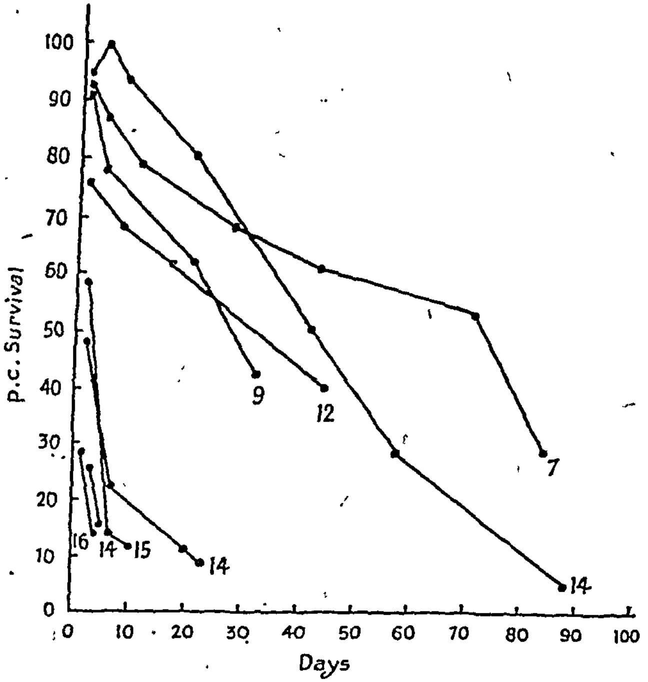

FIG. 7.—Sucrose (XIII).

and the erythrocytes become spherocytic, and are then, according to him, half-way to hæmolysis. The development of lysolecithin can be prevented by keeping cells and plasma in contact—by agitating the blood, for instance. A simple experiment was devised to see whether, if stored blood were prevented from sedimenting, the survival of the cells after a given time would be improved.

Blood was taken into the standard citrate-glucose mixture and the bottles were placed in a refrigerator in the ordinary way. During the first 24 hours the bottles were inverted and mixed several times; on each of the next four days they were mixed several times; on each of the next four days they were mixed and inverted every 12 hours, and thereafter once a day. This simple treatment did not completely prevent sedimentation, but it ensured a very much greater degree of contact between cells and plasma than usually occurs during storage.

The hæmolysis which occurs when blood is stored with high concentrations of glucose (E, see fig. 8) is less than with low concentrations (D, fig. 8). This is true whether small or large relative volumes of glucose solution are added to blood provided that the final concentration of glucose is high.

(2) *Osmotic Fragility Changes* (see fig. 9).—Osmotic fragility was found to increase progressively in blood stored with sodium citrate, I.H.T., and in heparinised and defibrinated blood. The increase in osmotic fragility occurred at a slightly slower rate in I.H.T. than in simple sodium citrate.

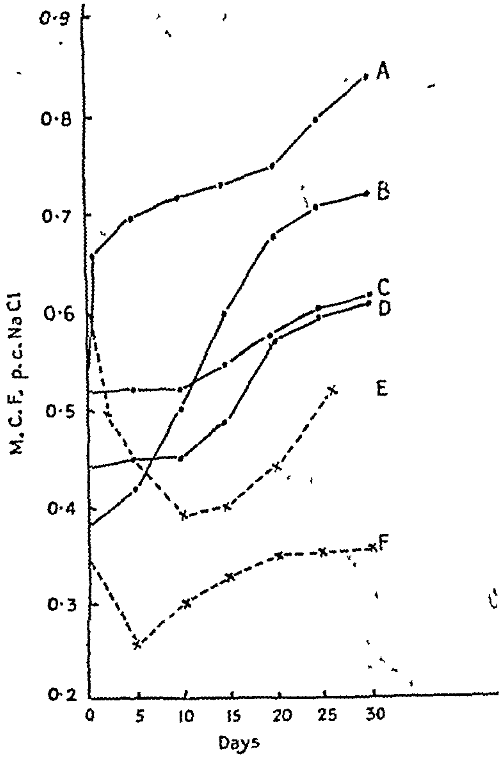

FIG. 9.—Osmotic fragility.
A = Rous-Turner (X).
B = sodium citrate (I).
C = citrate glucose (VII).
D = citrate glucose (VI).
E = Rous-Turner + final 4 per cent. sucrose.
F = sucrose (XIII).

The addition of a small amount of glucose to sodium citrate results in a diminution of the progressive increase in M.C.F. The addition of larger amounts increases osmotic fragility, however, and in the Rous-Turner solution the erythrocytes rapidly become very fragile to hypotonic saline solutions.

smallest increase is seen to occur after the transfusion of blood stored with the Rous-Turner solution where destruction of donor blood is minimal.

Hæmoglobinæmia was only observed on three occasions, once after the transfusion of blood stored for 28 days in the Rous-Turner solution (X), once with blood stored for 19 days in citrate-glucose (IX), and once with blood stored for 17 days in sodium citrate (I).

In vitro Tests.

(1) *Spontaneous Hæmolysis* (see fig. 8).—The preservative solution I.H.T. appears to be considerably more effective in preventing

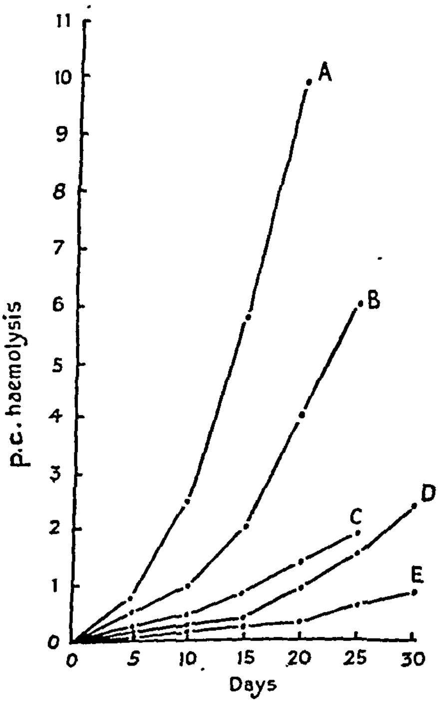

Fig. 8.—Spontaneous hæmolysis.

A, B = no glucose: A (I, III, IV); B (II).
C = dextrin, sucrose (XII, XIII).
D = glucose 0·6–1·0 per cent. (VI, VIII).
E = glucose 2·2–2·7 per cent. (VII, X).

hæmolysis than is a simple solution of sodium citrate. The effect of glucose in inhibiting the hæmolysis of stored blood is now familiar; it will be seen that sucrose is almost as effective as low concentrations of glucose in inhibiting hæmolysis. Dextrin was found to be as effective as sucrose in the particular concentration used (see Table I.).

(5) *Mean Corpuscular Volume* (*M.C.V.*).—In general, a progressive increase in cell volume was found in all solutions except nos. V and XI, which contain an excess of sodium citrate, and solution XIII and the other solutions containing sucrose.

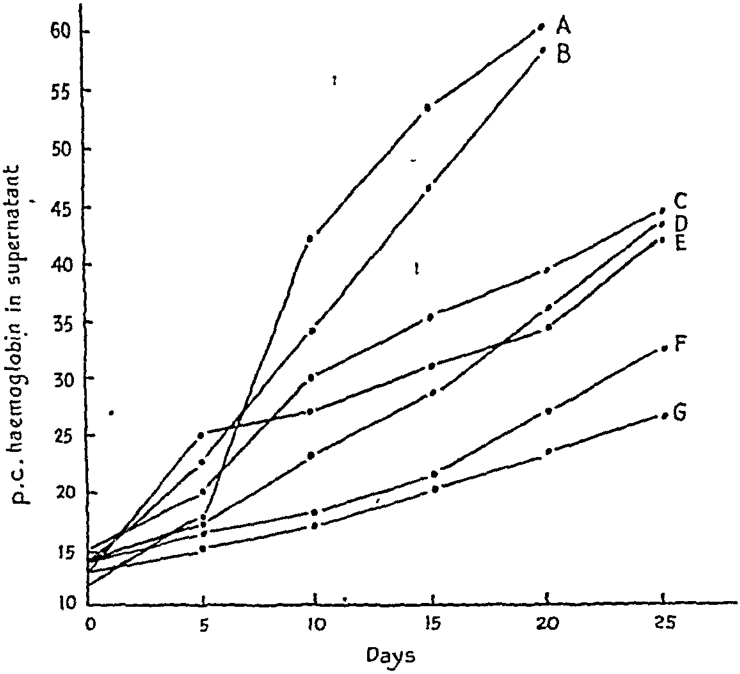

FIG. 10.—Mechanical fragility.
A = excess sodium citrate (V).
B = heparin (IV).
C = sucrose (XIII).
D = citrate, I.H.T. (I and II).
E = Rous-Turner (X).
F = citrate glucose (VI).
G = citrate glucose (VII).

At 8–13 days the average M.C.V. in a small group stored with sodium citrate (I) and I.H.T. (II) was 95 μ^3, and was slightly higher (104 μ^3) in a group of similar age of defibrinated and heparinised bloods (III + IV).

In the citrate-glucose mixtures M.C.V. was greater in the solutions which contained the greater relative volumes of glucose solution. Thus in a group stored for 16–21 days in solution VII it averaged 96 μ^3, whilst in a group stored for 18–20 days with solution IX it was 106 μ^3.

M.C.V. in solution V (excess citrate) was 66 μ^3 at 9 days, and in the similar solution containing a small amount of glucose (XI) was slightly higher (71 μ^3). Six samples stored in sucrose (XIII) for 7–16 days had an average M.C.V. of 68·5 μ^3.

Osmotic fragility was *diminished* in "excess" sodium citrate (solution V), although after 10–14 days' storage fragility started to increase. Sucrose, in a final concentration of 4·3 per cent., also reduces osmotic fragility. Similarly the addition of sucrose to citrate-glucose solutions results in a considerable decrease in osmotic fragility. When sucrose is added to the Rous-Turner solution, fragility first increases, then diminishes, then increases again (see fig. 9).

(3) *Effect on the Osmotic Fragility of Preliminary Treatment of the Erythrocytes with Fresh Plasma.*—Samples of blood stored for 11–13 days with three different solutions lacking glucose (III, IV, V) were tried. It was found that there was no significant reduction of the already increased osmotic fragility after the samples had stood for 1½ to 3 hours in fresh plasma. Blood stored in sucrose for 5 and 12 days also showed no change after treatment with fresh plasma, even after periods up to 24 hours. It will be remembered that in this solution osmotic fragility becomes lower than normal during storage. Samples stored for 11 and 19 days in solution XI, in which osmotic fragility is also found to become lower than normal, showed no alteration in M.C.F. after 3 and 9 hours in plasma or when treated with 0·86 per cent. NaCl.

Five citrate-glucose mixtures were next tested (VI, VII, IX, X, XI). Samples of blood stored for 14 and 21 days with solution VI showed a change in M.C.F. from 0·47 to 0·43 per cent. NaCl and from 0·50 to 0·42 per cent. NaCl respectively after 3½ hours in fresh plasma. The M.C.F. of a sample stored for 8 days in solution VII was reduced from 0·52 to 0·36 per cent. in 3½ hours. Samples stored from 18 to 20 days in solution IX showed an average change in M.C.F. from 0·70 to 0·48 per cent. NaCl at the end of 1 hour and to 0·40 per cent. NaCl at the end of 5 hours. Samples stored with the Rous-Turner solution for 21 days showed a change in M.C.F. from 0·71 to 0·45 per cent. NaCl in 1 hour. In the above experiments a slightly smaller reduction in M.C.F. occurred when the samples were placed in 0·86 per cent. saline instead of plasma.

Finally, a few samples of blood stored for much longer periods in citrate-glucose mixtures were tested. The M.C.F. of blood stored in solution VI for 32 days was reduced from 0·67 to 0·62 per cent. NaCl in 3½ hours, and that of blood stored in the Rous-Turner solution for 49 days was changed from 0·86 to 0·66 per cent. NaCl in the same period.

(4) *Mechanical Fragility.*—As shown in fig. 10, a progressive increase in mechanical fragility occurs during storage in all solutions. The figures in fig. 10 for citrate-sucrose (XIII) and for the Rous-Turner solution (X) differ slightly from those published previously [Mollison and Young, 1941]. This is due to the fact that the present estimations are based upon a larger number of samples and also because, owing to a mistake in the previous paper, an incorrect average figure was given for the sucrose samples.

erythrocytes were found surviving 5 weeks after transfusion; no details of the number of cases or the length of storage are given, however.

Wiener and Schaefer [1939] estimated the survival of stored citrated blood and found an inverse relationship between length of storage and length of survival. Blood stored for 6 days or less was not completely eliminated from the recipient's circulation for 9–15 weeks, but blood stored for more than a week was much more rapidly eliminated. This conclusion will be seen to agree closely with that reached from the present results. Rather shorter survival was found by Bushby *et al.* [1940], and very much shorter survival by Belk and Barnes [1941].

The falling off in survival after 6 days' storage that has been found by several workers may well be correlated with the virtual disappearance of glucose from stored citrated blood that occurs at about this time.

Solutions containing Glucose.—The survival of blood stored with citrate-saline-glucose and citrate-glucose mixtures has previously been estimated by Bushby *et al.* [1940], Maizels and Patterson [1940], and Mollison and Young [1940]. Bushby *et al.*, using 40 c.c. of 5 per cent. glucose to 440 c.c. blood, followed survival for 5–25 days after transfusion. They found that survival was much better than with stored citrated blood, but their estimate that only about 60 per cent. of the erythrocytes of blood stored for 8–14 days are surviving at the end of 5 days is distinctly lower than that described in the present paper. These workers do not explain how they calculate percentage survival, and it is possible that the method they use partially explains the difference in estimates. Alternatively, the shorter survival found by them may be due to the fact that they used a lower concentration of glucose.

Maizels and Patterson, using a citrate-saline-glucose solution (final concentration of glucose in blood mixture 1 per cent.), found more than 75 per cent. of donor erythrocytes surviving after 6 days and 45–100 per cent. after 15 days when blood stored for 7–14 days was used. In most cases survival was not followed to completion.

Mollison and Young [1940], using the same solution as Maizels and Patterson, found that the total time of life of erythrocytes transfused after 0–17 days' storage was at least 70–90 days and that, although there was some initial destruction of the older bloods shortly after transfusion, the majority of the cells survived normally.

Krüpe [1941] used a "direct" method of identifying donor cells with anti-M and N test fluids and found rather variable survival after the transfusion of blood stored for 1–9 days in a citrate-glucose mixture. Donor cells were identified at periods ranging from 9 to 44 days after transfusion, but in the majority of cases survival was not followed to completion.

The results of the present series of experiments with the standard citrate-glucose mixture are in close agreement, as to the total time

(6) *pH Changes.*—The pH changes observed in blood stored with some of the preservative solutions have been represented graphically in fig. 11. A progressive fall occurs during storage in all solutions except dextrin. pH is lowered in the solutions containing glucose, and

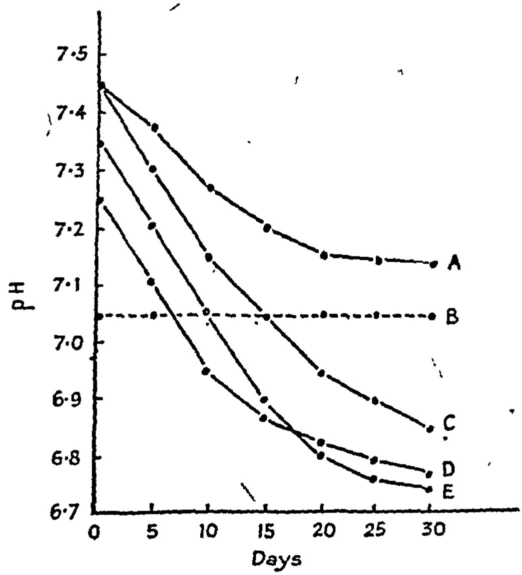

FIG. 11.—pH changes.
A = citrate (I).
B = dextrin (XII).
C = citrate glucose (VI).
D = sucrose (XIII).
E = Rous-Turner (X).

is lower in the Rous-Turner solution (X) than in the standard citrate-glucose mixture (VI). The changes in the citrate-sucrose solution (XIII) are similar to those in the Rous-Turner solution, although pH is initially lower in the former.

DISCUSSION.

In vivo Survival Tests.

In the case of most of the solutions used it is not possible to compare the results of the *in vivo* survival tests with those of other workers because no previous estimates have been published. Several workers have, however, estimated the survival of blood stored in citrate or citrate-saline mixtures, and a few workers have used solutions containing small amounts of glucose.

Solutions lacking Glucose.—Vlados [1934] in a paper on the solution I.H.T. mentions that the Ashby method was used and that some

stances. Firstly, when hæmolysed stored blood is transfused, sufficient free hæmoglobin might be injected to produce hæmoglobinæmia even if no immediate destruction of donor cells occurred *in vivo*. Secondly, if blood is appreciably hæmolysed by being brought into contact with fresh compatible plasma, its transfusion would be expected to result in an approximately equal degree of intravascular hæmolysis *in vivo*. Thirdly, the transfusion of aged erythrocytes might be followed by intravascular breakdown by mechanical forces.

In practice it is found that only the first and second possibilities occur. That is to say, hæmoglobinæmia only follows when sufficient free hæmoglobin is injected or when blood that is hæmolysed by fresh plasma *in vitro* is transfused. In the second case, the *in vitro* test of bringing a sample of the donor blood into contact with fresh plasma at room temperature may give an exaggerated impression of the degree of hæmolysis to be expected in the recipient's plasma *in vivo*, because the osmotic fragility of erythrocytes is appreciably lower at 37° C. Thus in one of the cases transfused with the Rous-Turner solution, although 10–20 per cent. of the donor cells lysed in fresh compatible heparinised plasma at room temperature, no hæmoglobinæmia was noted although the transfusion was completed within 60 minutes. Nevertheless, it seems clear that one of the limitations to the amount of glucose that can be used in the storage of blood is the production of such a degree of hypertonicity in the erythrocytes that a proportion of them rupture on contact with the recipient's plasma. In the Rous-Turner solution it is apparent that this border-line is reached in some samples. However, as pointed out above, the solution used in the present experiments contained a slightly higher concentration of sodium citrate than the original solution, and this may possibly increase osmotic fragility slightly. In that case the hæmolysis in fresh plasma of erythrocytes stored in the Rous-Turner solution would seldom, if ever, occur with samples less than 28 days old.

From the results of *in vitro* experiments upon the effect on the osmotic fragility of erythrocytes stored in citrate-glucose mixtures, of allowing them to stand in fresh plasma, it would be expected that the majority of the erythrocytes of blood stored in the Rous-Turner solution for, say, less than 28 days would acquire a normal osmotic fragility *in vivo* after transfusion. Thus it is not surprising to find that the transfusion of such blood does not affect the curve of osmotic fragility of blood taken from the recipient immediately after transfusion. In 3 cases fragility was found to be precisely the same in samples taken before and after transfusion, although it could be shown that the latter sample contained considerable numbers of donor cells.

Even when destruction is very rapid, as after the transfusion of blood stored without glucose for more than 12 days for instance, it can be shown to be almost entirely extravascular and the disappearance

of survival of the transfused erythrocytes, with the observations of Mollison and Young [1940] upon the survival of blood stored in a citrate-saline-glucose mixture. In the present series, however, rather more attention has been paid to the phase of destruction that occurs immediately after transfusion as it is clear that, although the total time of survival of a given sample of stored blood may be little less than normal, the value to the recipient may be much reduced if the normal percentage of transfused erythrocytes do not survive during the period immediately following transfusion.

By taking a sample from the recipient immediately after a relatively rapid transfusion before appreciable destruction has occurred, the small degree of destruction that takes place in the next few hours is more clearly appreciated, and a more accurate estimate of the percentage of cells that survive subsequently is obtained. This point is emphasised in fig. 4, from which it will be seen that although the total time of survival of blood stored for 11–15 days in the standard citrate-glucose solution is virtually as long as that of fresh blood, the percentage survival at intervals after transfusion is definitely lower in the former group and the blood is thus of slightly less value to the recipient.

In vivo survival was not improved by increasing the contact between cells and plasma during storage, that is to say by conditions which should, according to Fåhraeus' theory, have appreciably diminished lysolecithin formation. Lloyd [1941] has shown that hæmolysis occurs in kept blood even when lysolecithin formation is almost completely prevented.

Bilirubin Changes.—Although the relationship between blood destruction and an increase in serum bilirubin shows clearly when the cases are averaged, great variations are found if the cases are considered separately. This is to be expected, since an increase in serum bilirubin depends upon many factors, of which the degree of destruction of donor blood is only one. Other factors are the exact time after transfusion at which the sample is taken, the amount of blood given in relation to the blood-volume of the recipient, the efficiency of the liver in clearing the plasma of bilirubin, and destruction of the *recipient's* erythrocytes. (It has recently been shown by Aubert [1942] that the transfusion of serum or plasma of group O to recipients of group A often causes a rise in serum bilirubin, together with other signs of blood destruction.)

The Occurrence of Hæmoglobinæmia and the Fate of "Fragile" Cells after Transfusion.—As mentioned above, hæmoglobinæmia was encountered more often (2 cases) after the transfusion of blood stored in the "best" solutions (IX and X) than after the transfusion of blood stored without glucose (1 case), although survival was so much inferior in the latter.

Hæmoglobinæmia might be expected to occur in various circum-

reduced. Conversely, blood stored in the Rous-Turner solution shows very little hæmolysis despite the great swelling and increased osmotic fragility of its erythrocytes. As Maizels and Whittaker point out, degeneration of the red-cell membrane may also be important in determining the onset of hæmolysis in stored blood, and it seems likely from these observations that in some solutions it is the more important factor.

(2) *Osmotic Fragility.*—The figures for the sodium-citrate solution are very similar to those noted by other workers using citrate and citrate-saline mixtures [Crosbie and Scarborough, 1940; Bushby *et al.*, 1940; Dubash *et al.*, 1940]. The slightly slower rate of increase in osmotic fragility found in blood stored with I.H.T. agrees with the observations of Bagdassarov [1937].

Many workers [Bushby *et al.*, 1940; Dubash *et al.*, 1940; Denstedt *et al.*, 1940] have noted that the addition of small amounts of glucose to sodium-citrate solutions slows the progressive increase in osmotic fragility that occurs during storage, whereas the addition of larger amounts increases osmotic fragility, as also noted by Wilbrandt [1940]. On the basis of these observations, these authors have recommended solutions containing small amounts of glucose.

The observation that erythrocytes stored in the Rous-Turner solution rapidly become very fragile to hypotonic saline solutions has not apparently been made before. Willenegger and Ottensooser [1940] found scarcely any increase in osmotic fragility during storage with this solution, but they washed the corpuscles before estimating fragility. The effect of allowing erythrocytes stored in the Rous-Turner solution to stand in fresh plasma has been discussed previously [Mollison and Young, 1941], and the whole question of the significance of the osmotic fragility of erythrocytes stored in citrate-glucose solutions will be discussed again below.

In considering the significance of changes in the osmotic fragility of stored erythrocytes, several factors must be taken into account. Osmotic relationships, changes in cell shape, and alterations in the permeability of the red-cell membrane all play a part.

Although the red-cell membrane is normally impermeable to cations, potassium diffuses out of the red cell during storage [Dulière, 1931], and there is a coincidental inward diffusion of sodium as shown by a decrease in plasma sodium [Jeanneney *et al.*, 1939]. These changes begin within 30 minutes of drawing blood [Downman, Oliver, and Young, 1940] and are well advanced by the end of 5 days. At this time it seems likely that the total base content of the stored erythrocyte is not greatly altered because osmotic fragility is scarcely greater than normal (see fig. 9). Up to this time at least, therefore, an increase in corpuscular sodium is probably balanced by a decrease in corpuscular potassium.

Cell hæmoglobin, however, exerts a greater osmotic pressure than

from the circulation of the equivalent of, say, 300 c.c. of donor blood in a few hours can be observed without any accompanying hæmoglobinæmia. Vaughan [1942] has demonstrated that methæmalbumin [Fairley, 1940] cannot be detected in the recipient's plasma after the transfusion of stored blood, and this finding excludes the occurrence of all but small degrees of intravascular hæmolysis.

In vitro Tests.

(1) *Spontaneous Hæmolysis.*—The majority of the results are merely confirmatory of earlier work. The effect of I.H.T. in delaying the hæmolysis of stored blood was first described by Bagdassarov [1937]. The much more pronounced effect of glucose was first noted by Rous and Turner in 1916 and has since been confirmed repeatedly. Rous and Turner also noted that sucrose is almost as effective as glucose in delaying hæmolysis. Maizels and Whittaker [1940 *a*] have claimed that dextrin is more effective than glucose in inhibiting the hæmolysis of stored blood, and have suggested the use of dextrin as a preservative when it is desired to keep blood for long periods. In the present experiments, however, dextrin was not found to be as effective as high concentrations of glucose in inhibiting hæmolysis *in vitro,* and, as mentioned above, was found to have a far less favourable effect than glucose upon subsequent *in vivo* survival.

From the results it is clear that solutions which delay hæmolysis *in vitro* do not necessarily have a comparable effect upon *in vivo* survival. Bushby *et al.* [1940] pointed out that absence of hæmolysis in a sample of blood stored in citrate glucose did not necessarily imply good *in vivo* survival, and Maizels and Whittaker [1940 *b*] considered that a substance which delays hæmolysis *in vitro* might not have much effect upon *in vivo* survival.

A comparison of the results obtained with citrate-glucose mixtures VI and VII lends further emphasis to this point. The latter solution contains approximately four times as much glucose as the former and is considerably more effective in delaying hæmolysis. Nevertheless, *in vivo* survival of erythrocytes stored with the latter solution is no better than that of erythrocytes stored with the former.

In connection with the spontaneous hæmolysis that occurs in blood during storage, Maizels and Whittaker [1940] suggested that this was mainly due to the progressive swelling and final bursting of the erythrocytes. Blood stored in sucrose, however, starts to hæmolyse at a time when its erythrocytes show no trace of hæmolysis in 0·50 per cent. NaCl, and exhibits as much as 2 per cent. spontaneous hæmolysis when its erythrocytes still show no trace of hæmolysis in isotonic saline. The findings with the solution containing an excess of sodium citrate (V) are almost identical, and with both these solutions mean cell volume is

This might be explained in one of two ways. Either the osmotic pressure may be greater in the plasma-diluent phase of blood stored with solution VII, and this may hinder the free passage of glucose into the red cell, or, assuming that the glucose concentration in the water phase of the red cells is the same after a given period in both cases, the greater cell volume of erythrocytes stored in solution X might be responsible for their greater fragility to saline solutions, since when the tests are made water probably enters the red cells more rapidly than glucose can escape from them. There is an approximate relationship between the M.C.F. of the erythrocytes in different citrate-glucose mixtures and their subsequent *in vivo* survival.

If the erythrocytes stored with solutions VII and X contain the same concentration of glucose (see above discussion), the superior survival of those stored in solution X might be due to their greater size and consequently to the greater absolute amount of glucose that they contain. One difficulty in accepting this explanation lies in the fact that when sucrose is added to solution X during storage the mean cell volume of the corpuscles is much reduced, together with the osmotic fragility, at a certain phase of storage, but subsequent survival *in vivo* does not seem to be significantly affected.

At all events it seems clear that the action of glucose is chemical rather than physical. This was emphasised by Rous and Turner, who pointed out that one action of sugars was their ability to retard proteolytic digestion. Maizels [1941 *b*] has suggested that the inhibition by glucose of the release of organic phosphates may be a factor in prolonging survival.

(3) *Effect on Osmotic Fragility of Treatment with Fresh Plasma.*—Maizels and Patterson [1940] have pointed out that when stored erythrocytes are placed in fresh plasma, a loss of sodium from the erythrocytes cannot take place by a simple physical process in view of the high sodium content of normal plasma. Therefore, alterations in osmotic fragility occurring during storage that are not affected by treatment of the erythrocytes with fresh plasma are probably due to alterations in the base content of the erythrocytes. Alterations in osmotic fragility that are reversed by treatment of the erythrocytes with fresh plasma, on the other hand, may be assumed to be due to the presence in the erythrocytes of diffusible osmotically active substances in a concentration greater than that in fresh plasma.

With these considerations in mind it seems likely that the reduced osmotic fragility of erythrocytes stored in solutions V and XIII, and the increased osmotic fragility of erythrocytes stored for 11 to 13 days in solutions III, IV, and V, are due to alterations in the base content of the erythrocytes. The increased osmotic fragility of erythrocytes stored in citrate-glucose mixtures, on the other hand, seems likely to be due to the presence of glucose in the erythrocytes since treatment

plasma protein, so that after a time salts continue to enter the red cell without a corresponding outward diffusion of potassium. This suggestion was put forward by Maizels and Whittaker [1939] to explain the increase in osmotic fragility and mean cell volume that they found in erythrocytes stored with a citrate-saline mixture.

When blood is stored with sucrose, to which the red-cell membrane is impermeable, presumably the osmotic pressure of the sucrose more than counteracts that of the cellular hæmoglobin [Wilbrandt, 1940], so that salt may actually be lost from the red cell and osmotic fragility is found to be lower than normal. The low osmotic fragility of blood stored with a large volume of sodium citrate is difficult to explain.

When blood is stored in certain citrate-glucose mixtures, changes in osmotic fragility may occur much earlier and may be of far greater extent. In the case of the Rous-Turner preservative solution, for instance, median corpuscular fragility rises to 0·66 per cent. NaCl in 6 hours from the time of bleeding. Such a rapid increase in osmotic fragility cannot be due to an increase in corpuscular sodium, because sodium only diffuses slowly into the red cell, and, moreover, no such increase in M.C.F. is found in erythrocytes stored with a similar concentration of sodium citrate but without glucose.

It seems certain that the rapid increase in osmotic fragility of blood stored in the Rous-Turner solution is due to the diffusion of glucose into the red cell, a process which is known to take place fairly rapidly [Klinghoffer, 1940]. Maizels [1941 *a*] also concluded that the presence of glucose in red cells might increase osmotic fragility. It is to be expected, then, that the median corpuscular fragility of erythrocytes stored in citrate-glucose mixtures would give a measure, other factors being equal, of the amount of glucose that they have taken up.

Since glucose diffuses into human erythrocytes so readily, the term "isotonic" when applied to glucose solutions is misleading. Rous and Turner [1916] believed that human erythrocytes were impermeable to all sugars and chose an isotonic solution of glucose. DeGowin, Harris, and Plass [1940], modifying the Rous-Turner solution by reducing the amount of sodium citrate, also chose 5·4 per cent. glucose because they found that concentration to be isotonic with human serum according to depression of freezing-point experiments.

When glucose solutions are added to blood it is true that they will either lead to swelling or shrinking of the erythrocytes according as their concentration is low or high; but it is important to realise that conditions are progressively modified by the diffusion of glucose into the red cells.

It was found that although in general M.C.F. varied directly with the concentration of glucose in the mixture, osmotic fragility was much greater in solution X than in solution VII (see fig. 3), although both solutions contain approximately the same final concentration of glucose.

it will be seen that erythrocytes stored with solution V are also more fragile to shaking than erythrocytes stored with the Rous-Turner solution. DeGowin *et al.* also tested blood stored with a small relative volume of citrate, but were unable to compare the results with those obtained with the Rous-Turner solution because of the greater concentration of erythrocytes in the former and because no standardisation of the concentration of blood used for the test was attempted. Their conclusion that erythrocytes stored in a dextrose-citrate mixture resist shaking better than do those stored in sodium citrate alone does not seem to us to be a justifiable deduction from the evidence presented.

(5) *Mean Corpuscular Volume* (*M.C.V.*).—An increase in M.C.V. during storage in citrate-saline and citrate-saline-glucose mixtures was noted by Maizels and Whittaker [1940 *a*], and Crosbie and Scarborough [1941] found a progressive measure in the M.C.V. of blood stored with 3·8 per cent. sodium citrate.

As noted above, dilution of blood with *excess* 3 per cent. sodium citrate alone was found to reduce mean cell volume, whereas the addition of solutions of glucose produced an increase. In considering the effect on cell size of the addition of different amounts of sodium citrate and glucose solutions to blood, the volume of glucose solution is probably more important than the total volume of citrate-glucose mixture in relation to the volume of blood.

Although when glucose solutions are added to blood, cell swelling will vary with the relative volume of glucose solution added, Klinghoffer has shown that when a certain concentration of glucose is exceeded, the red-cell membrane becomes relatively impermeable to glucose, so that when, for example, increasing proportions of 5·4 per cent. glucose are added to blood the increase in volume is found to be less than would occur if water were added instead of glucose.

(6) *pH Changes.*—The initial figures for blood to which citrate has been added (7·45) and to which dextrin and glucose have been added (slightly lower) agree well with the observations of Maizels and Whittaker [1940 *b*]. The progressive fall in pH occurring in blood stored with citrate-glucose solutions was described by DeGowin, Harris, and Plass [1940], although their pH figures are somewhat higher than those given here.

Conclusions.

The results of the commonly accepted *in vitro* tests, made upon samples of stored blood, are fallacious indicators of the state of preservation of biological activity, as judged by the subsequent *in vivo* survival of the erythrocytes. In the selection of preservative solutions, however, *in vitro* tests may have a negative value. For instance, if blood hæmolyses rapidly in a given solution, that solution may safely be rejected. If, however, a solution is found to be successful in inhibiting

with fresh plasma restores osmotic fragility to normal. The older samples of blood stored in citrate-glucose mixtures were not altered in the same way by treatment with fresh plasma. Although it is possible that osmotic fragility could have been further reduced in these cases by the use of a greater relative volume of fresh plasma, it seems likely, when the results are compared with the previous ones, that these changes are no longer completely reversible *in vitro*. It is possible that changes in osmotic fragility which cannot be reversed *in vitro* are correlated with impaired *in vivo* survival.

Maizels and Patterson [1940], using a citrate-saline mixture containing a final concentration of 1 per cent. glucose, showed that the stored cells survived in the recipient's circulation after transfusion, but lost their extra sodium *in vivo* within 24 hours of transfusion. They found a big increase in the mean corpuscular sodium of blood taken from the recipient immediately after the transfusion, but noted that the figure had almost returned to normal by the end of 24 hours. In a series of 7 transfusions we have been able to confirm these results.

As mentioned above, however, an increase in corpuscular sodium does not necessarily imply an increase in corpuscular fragility (it may be compensated by a diminution in corpuscular potassium), so that these results are no evidence against the view that an increase in corpuscular fragility that is not reversible *in vitro* is correlated with impaired *in vivo* survival.

(4) *Mechanical Fragility*.—It was pointed out by Rous and Turner in 1916 that the mechanical fragility of the erythrocytes of different animals bears no constant relationship to the osmotic fragility. From fig. 10 it is clear that this lack of relationship between osmotic and mechanical fragilities also obtains with human erythrocytes stored in different solutions. Thus, for instance, despite the great difference between the osmotic fragility of erythrocytes stored in citrate-sucrose (XIII) and in the Rous-Turner solution (X), it will be seen that there is very little difference between the mechanical fragility of the two bloods. It will further be noted that there is no correlation between the amount of spontaneous hæmolysis that occurs in blood stored with a given solution and the mechanical fragility of the same erythrocytes. For instance, although blood stored with sodium citrate hæmolyses much more rapidly than blood stored with the Rous-Turner solution, the erythrocytes of the latter are, if anything, more fragile when shaken vigorously. Finally, it will be apparent that the results of these tests are in no way correlated with those of the *in vivo* survival tests.

DeGowin, Harris, and Plass [1940] found that erythrocytes stored with a dextrose-citrate solution resembling the Rous-Turner solution were more resistant to shaking than erythrocytes stored in a mixture of similar proportions but in which the glucose was replaced by saline. The latter solution thus somewhat resembles our solution V. *In fig. 10*

It may be emphasised that of the very many solutions that have been recommended for the preservation of blood, almost all up to now have been selected on the basis of *in vitro* tests. Such solutions are accordingly only by chance the best for preserving the biological value of stored erythrocytes.

In the selection of new preservative solutions, and particularly in devising new citrate-glucose mixtures, it is recommended that the effect of the solutions upon the survival of the erythrocytes after transfusion be used as the main criterion of value, in preference to the commonly accepted *in vitro* tests.

We should like to thank Dr. J. F. Loutit for permission to carry out this work; Dr. M. Maizels for helpful criticisms; Dr. G. L. Taylor of the Galton Laboratories Serum Unit, for very kindly supplying M and N test sera once more, and Dr. L. F. Hewitt for carrying out the pH determination.

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hæmolysis, it will not necessarily succeed in prolonging *in vivo* survival.

The degree to which stored erythrocytes resist vigorous shaking in the presence of glass beads is not correlated with their survival time *in vivo.*

Although the osmotic fragility of stored erythrocytes, as usually measured, has led to many fallacious conclusions as to the value of different blood preservatives, a revised test in which the erythrocytes are first treated with fresh plasma may be found more useful.

The *in vivo* survival of blood stored without carbohydrate is almost as good as that of fresh blood when the period of storage is 6 days or less. I.H.T., sodium citrate, and heparin all seem to have a similar effect upon subsequent survival; defibrinated blood seems to survive slightly better. In the treatment of anæmia it is suggested that the limit of 6 days is not exceeded when using blood stored in one of these solutions. *Blood stored for 14 days without carbohydrate is almost useless to the recipient as a source of biologically active erythrocytes,* although it usually does not contain sufficient free hæmoglobin at this time to be actually a source of danger.

Dextrin and sucrose, although they delay the onset of hæmolysis *in vitro* almost as well as glucose, have very little effect in prolonging the *in vivo* survival of blood.

The effect of glucose in prolonging the survival of erythrocytes is very definite, and varies with alterations in the amount and concentration of glucose that is added. When approximately one volume of citrate-glucose solution is added to four volumes of blood, variation of the concentration of glucose in the final mixture from 0·6 to 2·2 per cent. makes little difference to subsequent survival. If a larger volume of citrate-glucose solution is added, however, and a final concentration of 2·7 per cent. in the final mixture is attained, survival is very definitely prolonged. Thus, whereas blood stored in the standard citrate-glucose solution (VI) for periods of 11–15 days already shows some falling off in survival, blood stored with the Rous-Turner solution (X) for periods up to 21 days has a survival which is very little inferior to that of fresh blood. Although the Rous-Turner solution is thus a better preservative than the citrate-glucose solution in general use, the advantage is not obvious with blood stored for less than 10 days at least. Moreover, the large relative volume of diluent in the Rous-Turner solution is a definite disadvantage both from the administrative and transfusion points of view. Thus for blood banks in which the turnover of blood is completed in 10 days or less there is no advantage in changing to the Rous-Turner solution. Further, the use of a small volume standard citrate-glucose solution leads to the production of plasma with a relatively high protein content, and this is important when blood that is not required for transfusion is to be used for plasma production.

THE EFFECT OF REMOVAL OF THE POSTERIOR LOBE OF THE PITUITARY ON THE INHIBITION OF WATER-DIURESIS BY EMOTIONAL STRESS.

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Rydin and Verney [1938] showed that when water-diuresis in the dog was checked by emotional stress, the inhibition occurred in apparently unaltered form when the renal (see also Theobald [1934], Theobald and Verney [1935]) and suprarenal nerves had been divided, and whether or no this denervation were combined with section of the splanchnic nerves or even with decentralization of the entire abdominal sympathetic system. Measurement of the arterial pressure during the inhibition of urine-flow occurring under these different conditions suggested that the inhibition was not dependent upon a fall in blood-flow through the kidney, a deduction which received strong support in the rapid recovery of diuresis from the inhibition induced by temporary obstruction of the arterial blood-supply by means of a compression-unit previously implanted at the origin of the renal artery. These observations, together with others concerned with the effects of intravenous injections of adrenaline, indicated that local hæmodynamic events were not essentially related to the inhibition, and led to the conclusion that its agent was humorally conducted to the kidney and was not adrenaline. Furthermore, the finding that the inhibition by emotional stress resembled in time-course, degree, and in the accompanying changes in urinary chloride and nitrogen, that produced by a suitable intravenous dose of post-pituitary extract, suggested that the agent liberated in emotional stress was the substance to which the antidiuretic activity of post-pituitary extract is due. This suggestion we have now put to the test of direct experiment, and in so doing we have been greatly helped by the recent work of Pickford [1939], in which was described an inhibition of water-diuresis by the intravenous injection of acetylcholine in the atropinized dog, and its abolition by the removal of the posterior lobe of the pituitary demonstrated.

Methods.

Fully grown bitches were used in all the experiments. At a preliminary operation they were perineotomized in order to make

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by Messrs Burroughs Wellcome under the name "Infundin" and standardized to contain 10 international oxytocic units per c.c. The same batch was used throughout the experiment on any one dog, and the term *milli-unit* as used in this paper signifies the antidiuretic activity of 10^{-4} c.c. of the extract.

Removal of the posterior lobe of the pituitary was effected through the mouth under "nembutal"[1] anæsthesia. Aschner's [1912] diasphenoid route was adopted, and the method used was that described by Pickford [1939], with the exception that a tracheal cannula was not inserted. We found it an advantage to use an electrically operated dental drill to remove the inner table of the sphenoid.

When the necessary observations had been made, the animal was killed and the brain hardened *in situ* in the following way. The dog was anæsthetized either with ether or with chloralose (0·11 g. per kg. intravenously) and the head perfused through one carotid artery with saline at a pressure of 100 mm. Hg until the effluent from the contralateral external jugular vein was nearly clear. The fixative was then infused by the same cannula. In most experiments the fixative was 10 per cent. formalin in saline, but in later experiments a solution of 2 per cent. formalin and 5 per cent. glacial acetic acid in 80 per cent. alcohol was used. The second of these fixatives gave much better results with Bodian's [1936] method for staining nerve fibres. The region of the hypothalamus was dissected away with the overlying dura and scar, the tissue embedded in paraffin, cut in serial frontal or sagittal sections 10 μ thick at intervals of 0·05 to 0·1 mm. and stained with hæmotoxylin and eosin: in some cases Bodian's [1936] method for staining nerve fibres was used. Tissue removed at the operation for removal of the posterior lobe was fixed in the same fluids and examined in the same ways.

Results.

The effect of removal of the posterior lobe of the pituitary on the inhibition of water-diuresis by emotional stress.

In each dog before operation, and six animals have been used for this purpose, a strength of stimulus was found which consistently caused the rate of urine-secretion to fall approximately to the resting level. Fig. 1 illustrates the response. Forty-eight minutes after the dose of water had been administered the rate of urine-flow had risen to 3 c.c. per min. The stimulus was then applied for 1 min., and 7 minutes later the urine-flow had fallen to 0·1 c.c per min. After

[1] Sodium ethyl-methyl-butyl-barbiturate (Abbott). The drug was slowly injected intravenously in 5 per cent. aqueous solution in a dose of 33 mg. per kg. body-weight.

catheterization of the bladder simple. With the exception of the large animal used for the observations recorded in fig. 4, they weighed between 8·4 and 12·7 kg. They were fed each evening and allowed water *ad libitum*, the diet, except in the case of the large animal to which we have just referred, being 8–10 oz. of biscuit supplemented once a week by 8 oz. of raw meat.

Denervation of the kidneys was performed, under ether anæsthesia and with full surgical precautions, through a mid-line abdominal incision. The peritoneal attachments of each kidney were divided between a series of ligatures, the kidney was lifted from its bed and completely isolated apart from its arterial, venous, and ureteric connexions, and these structures were then carefully denuded of all visible nervous and connective tissue. In addition, the splanchnic nerves on each side were severed as they left the crus of the diaphragm. The lumbar sympathetic chain was then exposed on each side, and as much as possible of its upper part removed: usually the second, third, and fourth lumbar ganglia with the intervening parts of the chain were excised. Replacement of the kidneys into their natural positions was assured by tying together each pair of ligatures between which the peritoneal attachments of the kidney had been divided. All of the observations recorded in this paper, with the exception of those given in fig. 4, have been made on animals whose kidneys and suprarenals had been denervated by this procedure.

In any one animal water-diuresis curves of small variability were obtained in the following way. In the morning a dose of warm tap-water, varying from 250 to 300 c.c. according to the size of the animal, was given by stomach-tube, and 3 to 4 hours later the animal was placed in a Pavlov stand in a secluded room which was reserved for the observations with which this paper deals. The dose of water was then repeated and the response measured by collecting the urine continuously from a self-retaining catheter which conducted it to a series of graduated tubes. All comparative tests of the effects of emotional stress and of post-pituitary extract were made at the height of a diuresis produced in this way. More uniform results were obtained in any one dog by always making the injection or applying the stimulus at the same time after the dose of water, usually 40 to 50 minutes (see p. 399). Emotional stress was produced either by the sounding of an electric motor-horn near the animal, or by faradic stimulation through needle electrodes inserted into the subcutaneous tissue of the flanks, the strength of stimulus in each case being just enough to cause definite resentment. The two methods were equally effective in inhibiting water-diuresis, but with most dogs the second method was used. Injections of post-pituitary extract were made into the malleolar vein, the extract being suitably diluted with 0·9 per cent. NaCl so as to give the required dose in 0·5 c.c. The extract was that marketed

by Messrs Burroughs Wellcome under the name "Infundin" and standardized to contain 10 international oxytocic units per c.c. The same batch was used throughout the experiment on any one dog, and the term *milli-unit* as used in this paper signifies the antidiuretic activity of 10^{-4} c.c. of the extract.

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persisting at this low level for 10 minutes the flow gradually increased, and 45 minutes after the application of the stimulus it had recovered to the rate of nearly 2 c.c. per min. Fig. 2, A and B, show the effects

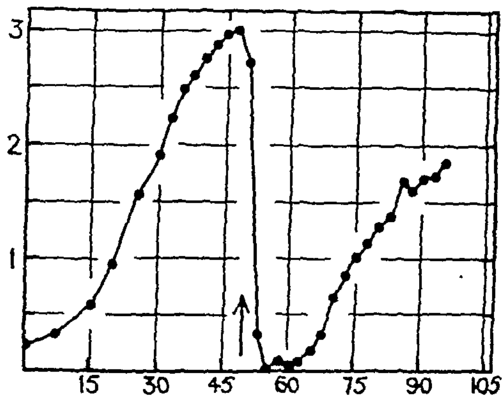

Fig. 1.—Inhibition of water-diuresis by emotional stress in a normal bitch: "Sin," weight= 9·7 kg. The dose of 300 c.c. of water was given at zero time, and times in minutes after the giving of water are plotted as abscissæ. Ordinates show the rate of urine-secretion over the interval before the plotted point, the intervals being usually 2·5 min. All graphs in this paper are plotted in this way. At the arrow the dog was excited for a period of one minute by a weak faradic current through needle electrodes in the flanks.

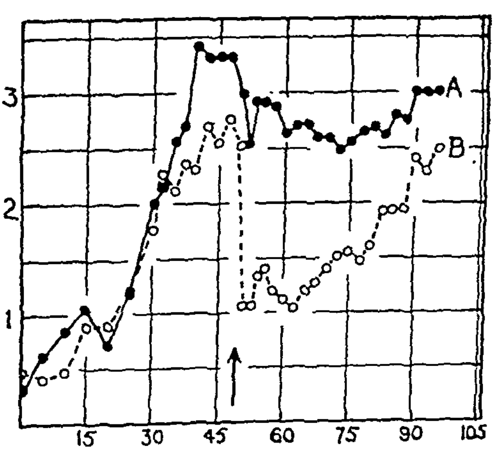

Fig. 2.—The effect of the same emotional stimulus, applied at the arrow, on the same bitch as in fig. 1, but 26 days (graph A) and 32 days (graph B) after removal of the posterior lobe. Ordinates and abscissæ as in fig. 1.

of a similar degree of emotional stress in the same animal 26 and 32 days after removal of the posterior lobe of the pituitary. In each instance the emotional disturbance was as great as that which caused before operation the large inhibition seen in fig. 1; but in the experi-

ment illustrated by the graph A of fig. 2 practically no inhibition resulted, and in that by the graph B the response, although quite large, was much less than that before posterior hypophysectomy. In each of the six animals the emotional stress which before operation caused large inhibitions gave, after posterior hypophysectomy, either no inhibition or diminished inhibitions not larger than that shown in B of fig. 2. With all six animals, however, such diminished inhibitions were seen in some of the tests after removal of the posterior lobe: of 94 tests after posterior hypophysectomy, 44 showed no inhibition and 50 a small but definite inhibition which was never larger than that given in B, fig. 2, and only approached this size in 10 of the tests. We conclude that the inhibition of water-diuresis by emotional stress is almost, but not completely, abolished by the removal of the posterior lobe of the pituitary. A paramount rôle, therefore, in the full expression of the inhibition is played by the posterior lobe, and the inference is compelling that under emotional stress a quantity of antidiuretic hormone is discharged from it.

Microscopic examination of the tissue removed at the operation of posterior hypophysectomy showed the posterior lobe apparently complete with the pars intermedia; but since the stalk of the pituitary and the infundibulum are regarded as being of the same structure as the posterior lobe [Fisher, Ingram, and Ranson, 1938], it is unlikely that all of the tissue capable of secreting or discharging antidiuretic hormone will be removed or destroyed by this operation alone. In this connexion it is of interest to recall Sato's [1928] finding that 0·25 per cent. acetic acid extract of the dog's tuber cinereum delays water-diuresis in the rabbit when the administration of the water is accompanied by the subcutaneous injection of the extract: the amount of antidiuretic substance in the extracts after hypophysectomy is stated by Trendelenburg [1928] to be increased. Four of our six animals were subjected to further operative interference in the pituitary region, but the remaining two were killed, the one 42 and the other 140 days after posterior hypophysectomy, and in each the anatomical picture revealed by serial section of the pituitary region was essentially the same. A mid-line sagittal section of this region in one of these animals is reproduced diagrammatically in fig. 3, *a*, and a similar section in a normal animal in fig. 3, *b*. The removal of the posterior lobe has left the stalk and infundibulum apparently intact, but the ventral wall of the cavity of stalk and third ventricle is rather thinner than in the normal; and the Bodian method of silver-staining for nerve fibres showed that the great mass of fibres which normally runs to the posterior lobe *via* the stalk was greatly reduced by loss of the posterior lobe. Many fibres, however, were still to be seen entering the stalk and running towards its blind end, first in definite bundles, but, more distally, these bundles were replaced by individual fibres running in

no common direction. With respect to the disposition of the nerve fibres and the nature of the cells, the remaining stalk resembled posterior lobe tissue: on anatomical grounds there is, therefore, reason to expect

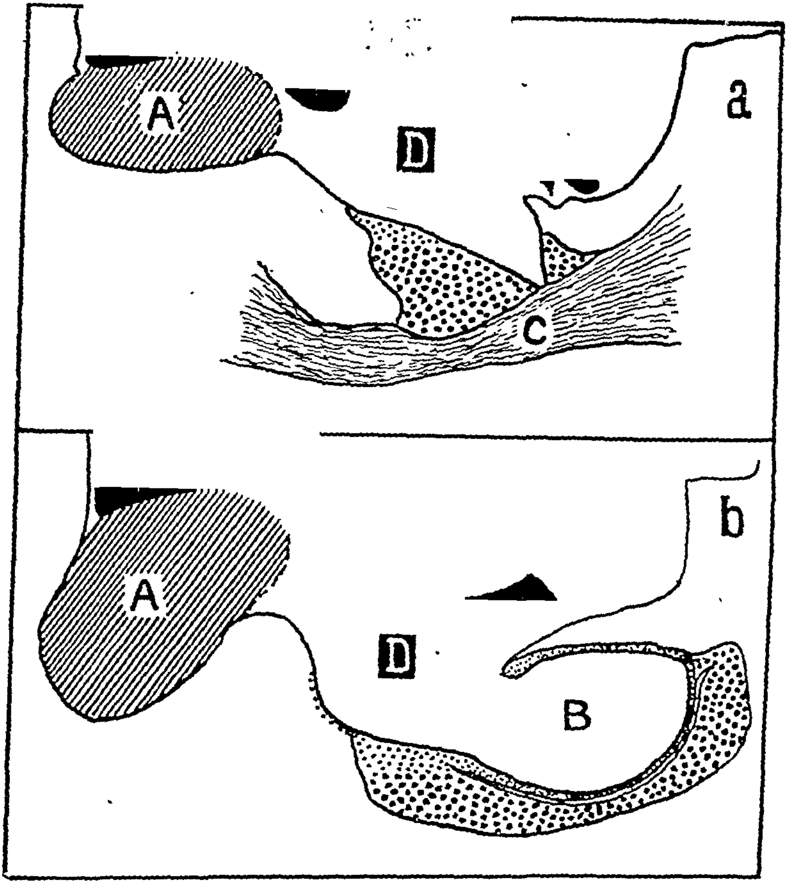

FIG. 3.—(*a*) Sagittal section, near mid-line, of the hypothalamic and hypophyseal regions after removal of the posterior lobe: "Tarzan," wt. = 11·2 kg. (*b*) Corresponding section from a normal dog. *A*, optic chiasma; *B*, posterior lobe; C, scar tissue; D, third ventricle. The heavy stipple indicates anterior lobe tissue. ×8.

a residuum of antidiuretic activity after removal of the posterior lobe.

In the dog the anterior lobe forms a covering over the whole of the posterior lobe, and in the operative removal of the posterior lobe it thus becomes necessary to split the ventral portion of the anterior lobe. There is, therefore, always some damage to the anterior lobe, but, as is seen in fig. 3, *a*, the portions of the anterior lobe in front of and behind the stalk are still intact and, with the lateral masses of

anterior lobe tissue, are in all probability quite adequate to maintain normal function. No portion of the pars intermedia could be seen in the sections from the animals after posterior hypophysectomy.

If the residuum of inhibition of water-diuresis by emotional stress after removal of the posterior lobe has its structural correlative in the remnant of neuro-hypophysis, the determination of the functional proportion of tissue removed at posterior hypophysectomy by comparing the post-pituitary extract equivalents of the full and residual inhibitions becomes of interest. Before proceeding to this determination, however, we shall give a series of responses to post-pituitary extract during water-diuresis, in order to disclose the indices which appear to give the most accurate measure of responses of different magnitude, and to emphasize the conditions which favour uniformity in response to a constant dose.

Water-diuresis in the dog as a means of assaying post-pituitary anti-diuretic activity.

The graphs in fig. 4 represent a series of nineteen experiments performed on a 15·8 kg. bitch.

The procedure in each instance was as follows. At 1 p.m. a preliminary hydrating dose of 300 c.c. of tap-water (T. 37°) was given by stomach-tube and the animal put in its kennel. An hour and a quarter later the animal was catheterized in a Pavlov stand, and ten minutes after the urine-flow had fallen to a rate of under 0·4 c.c./min. the test-dose of 300 c.c. of water was administered. The period between the two doses of water varied between 105 and 131 min., and the time at which the test-dose was given is zero time in the figures. At the end of each observation the animal was returned to its kennel and given 10 oz. of biscuit and half a pint of bread and milk. The experiments were intermitted one day each week, and on the evenings preceding these days the diet was supplemented by $\frac{3}{4}$ lb. of raw meat.

Fig. 4, *a*, gives the extreme and the mean rates of urine-flow in five simple responses to the test-dose of water: this mean is shown by the interrupted lines in all succeeding parts of the figure. Fig. 4, *b*, *c*, *d*, *e*, *f*, *g*, and *h*, give the responses to 0·25, 0·5, 0·75, 1·0, 2·0, 5·0, and 10·0 milli-units respectively, the doses being injected intravenously at the times shown by the arrows. No injection was given earlier than 30 min. after the dose of water, by which time absorption of the 300 c.c. of water by this animal was almost certainly complete (see Klisiecki, Pickford, Rothschild, and Verney [1933]). Fig. 4, *a*, shows that during the succeeding 15 min. the rate of urine-flow is maintained at a high and fairly uniform level, but since during this period the water-load of the animal will be rapidly falling, and the sensitivity to injected extract correspondingly increasing (see Hart and Verney [1934], Pickford [1936]), the importance of making the injections at the same time after the administration of the water will be evident. As a test of this, 0·5 milli-unit was injected on one occasion 40 min. instead of 30 min. after the test-dose of water: the minimum rate during the 15-minute period after the injection was 1·75 c.c./min. —definitely less than the minimum rates of the two responses given in fig. 4, *c* —and showed no appreciable recovery afterwards. There is, however, another factor which interferes with the reliability of this minimum rate as a

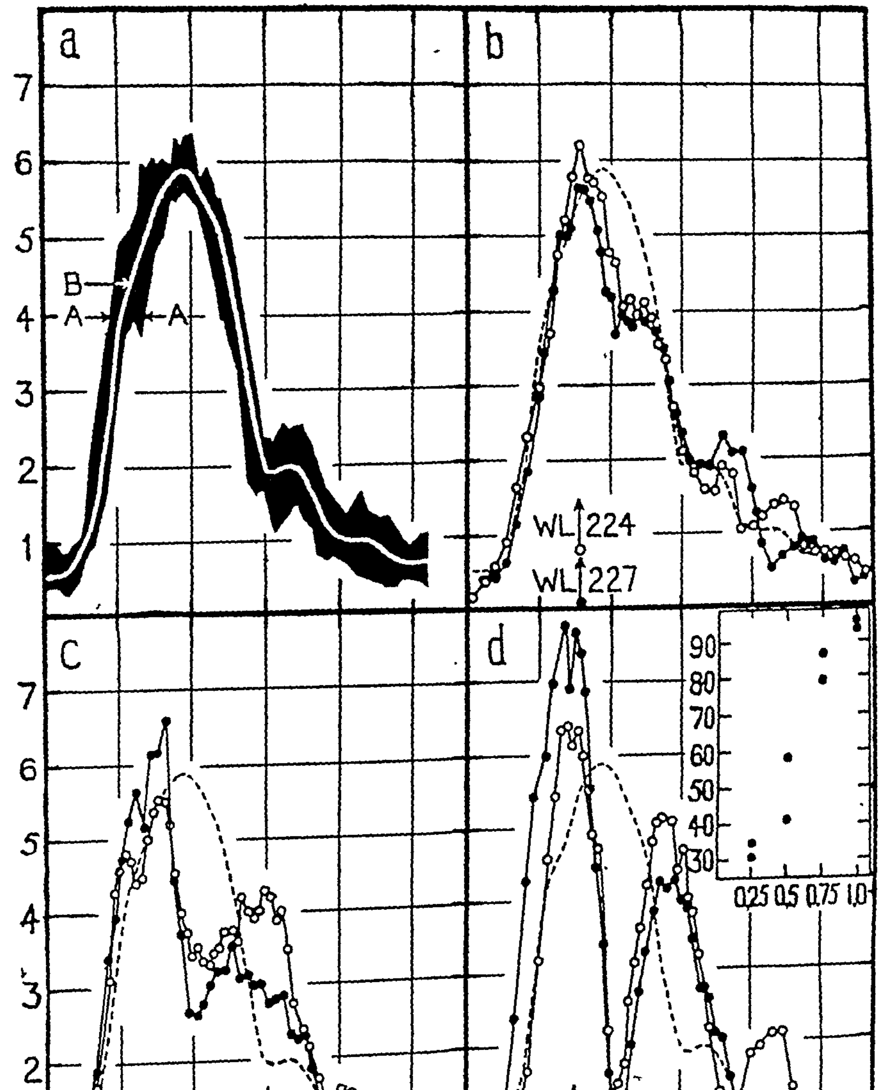

FIG. 4.—The responses to a series of doses of post-pituitary extract injected into a normal bitch weighing 15·8 kg. (*a*) The extreme (A) and mean (B) rates of urine-flow in five observations in which no extract was given. In three of these 1 c.c. of 0·9 per cent. NaCl was injected at time 30 and had no apparent influence on the course of diuresis. The mean (B) is reproduced in each subsequent part of the figure. (*b*) Two responses to 0·25 milli-unit injected at the times shown by the arrows. (*c*) Two responses to 0·5 milli-unit. (*d*) Two responses to 0·75 milli-unit.

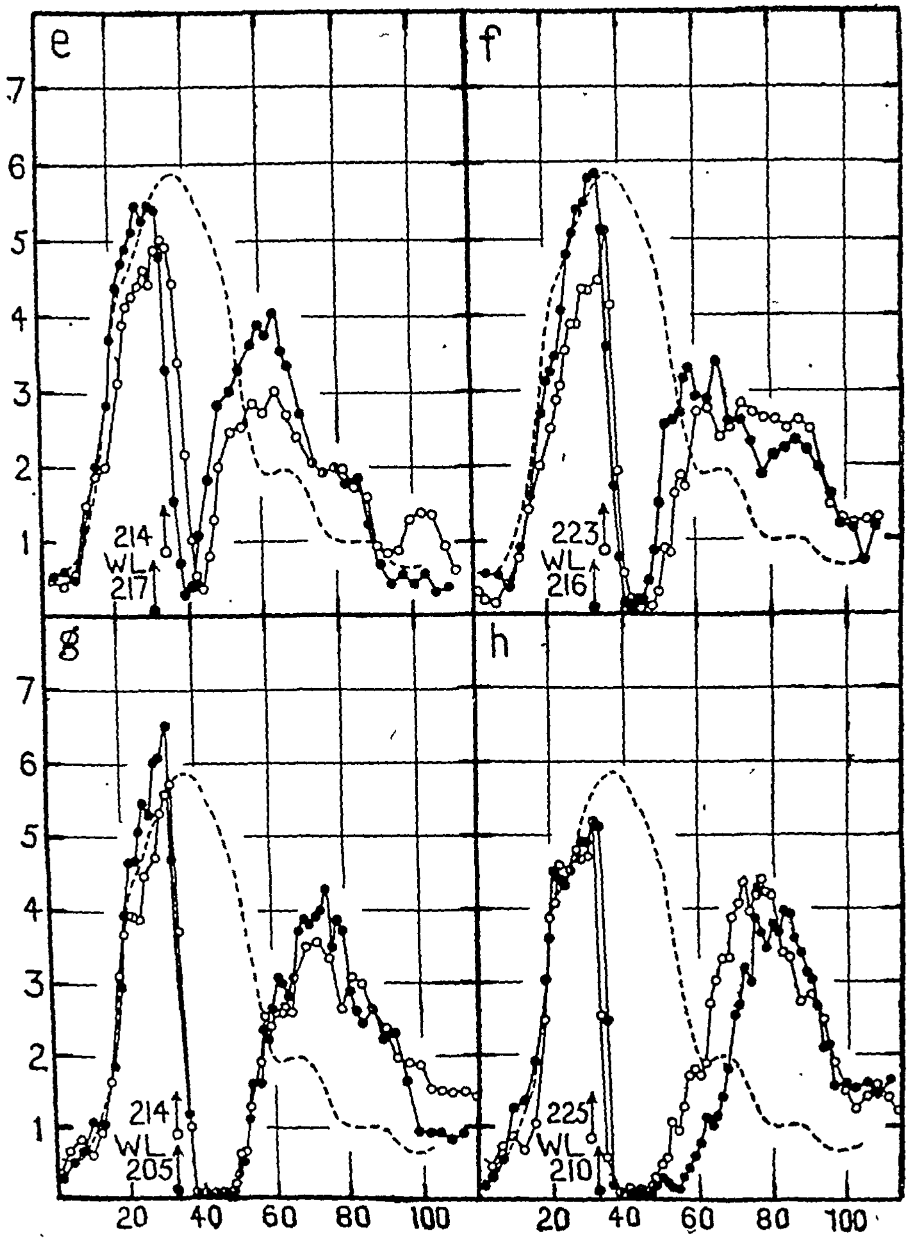

FIG. 4, *continued*.—(*e*) Two responses to 1·0 milli-unit. (*f*) Two responses to 2·0 milli-units. (*g*) Two responses to 5·0 milli-units. (*h*) Two responses to 10·0 milli-units. The difference between the volume of water given (300 c.c.) and the volume of urine excreted up to the time of the injection is expressed as water-load (WL): this difference is recorded against the arrow relating to the corresponding graph. Ordinates and abscissæ as in fig. 1. For further description see text.

measure of the response, namely, variability in the rate of urine-flow at the time when the injections are given. In one of the observations on the effects of 0·25 milli-unit (not included in fig. 4, *b*) the response to the test-dose of water showed an abnormally low maximum when the injection was given at 32 min. The minimum rate in the subsequent 15-minute period was as low as that in the larger of the two responses to double this dose in fig. 4, *c*. The percentage fall in rate, however, was definitely less, and was, indeed, approximately the same as the percentage falls in the other two responses to the same dose. While, therefore, the ideal conditions for assaying differences in post-pituitary antidiuretic activity would appear to be equality in period between the administration of the test-dose of water and the injection, and close parallelism between the courses of urine-flow during this period, the effect of small anomalies in the latter will be largely corrected if the percentage reduction in rate is taken as the index of the magnitude of the response. The percentage reductions in rate in all the experiments given in fig. 4, *b*, *c*, *d*, and *e*, are plotted against the corresponding doses as an inset in fig. 4, *d*.

With the precautions outlined one can, then, readily distinguish in this animal between adjacent effects in the series 0·25, 0·5, 0·75, and 1·0 milli-unit. With doses larger than that which reduces the urine-flow momentarily to the resting level—in this animal 1·0 milli-unit—the responses show an increasing lag in recovery with increasing dose. The mean period between the times when the descending and ascending limbs of the responses reach the 1·5 c.c./min. level is 7·5 min. with the doses of 1·0 milli-unit, 13 min. with those of 2 milli-units, 18 min. with those of 5 milli-units, and 27 min. with those of 10 milli-units; and the curves show that with few observations one may safely distinguish between these amounts of extract.

With these facts and considerations in mind we may now proceed to compare the post-pituitary extract equivalent of the inhibition produced by emotional stress before, with that produced after removal of the posterior lobe, with a view to estimating the functional proportion of tissue remaining after the operation.

The estimation of the functional proportion of neuro-hypophyseal tissue remaining after removal of the posterior lobe.

First it is necessary to estimate the amount of antidiuretic substance which is liberated in response to a standard period of emotional stress before the removal of the posterior lobe. As the size of the inhibition produced by any one dose of post-pituitary extract may change somewhat in the course of a long experiment, comparison of the inhibition by emotional stress with inhibitions by suitable doses of post-pituitary extract should be made over as short a period as possible. Fig. 5 illustrates an assay before removal of the posterior lobe, all of the six curves shown being obtained within a period of 8 days. In fig. 5, *a*, the inhibition produced by 40 seconds' faradic stimulation is compared with the inhibition produced by 2·0 milli-units injected intravenously at the same time on a similar curve: during the emotional stress more than 2·0 milli-units are liberated. In fig. 5, *b*, comparison is made

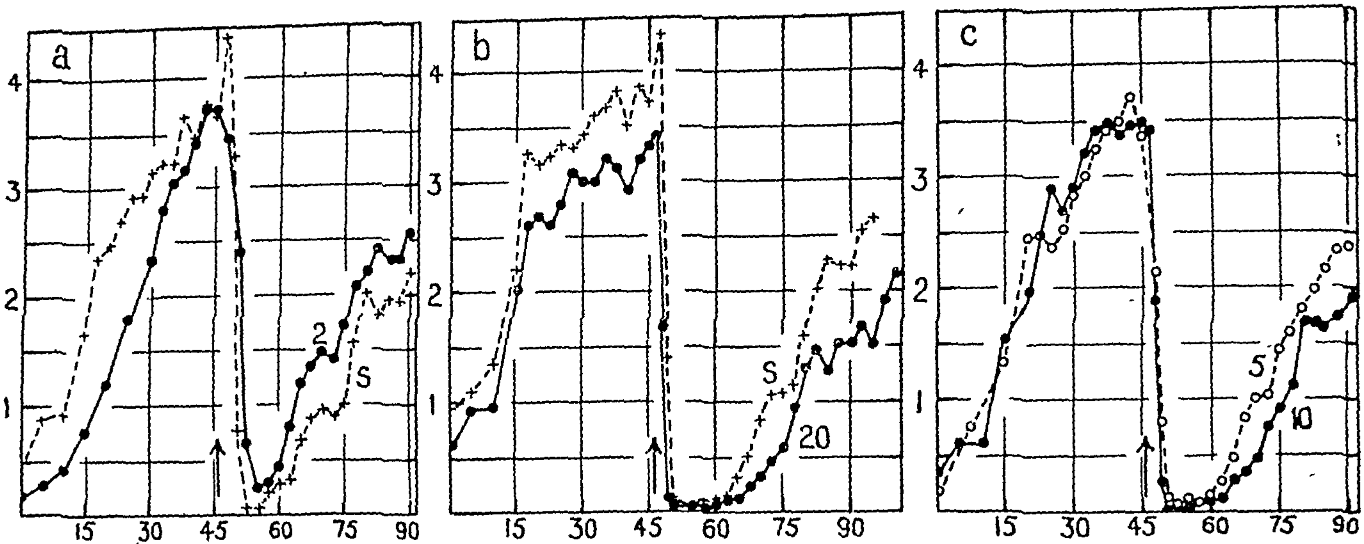

Fig. 5.—The estimation of the amount of antidiuretic substance liberated in response to 40 seconds' emotional stimulus before removal of the posterior lobe: "Tarzan," wt. = 11·2 kg. Dose of water = 300 c.c. The stimulus and injection were given at the arrow. (*a*) The inhibition produced by emotion (graph S) is compared with that resulting from the intravenous injection of *2 milli-units (graph 2)*. (*b*) Another inhibition from an equal stimulus (graph S) is compared with that from 20 milli-units (graph 20). (c) The inhibitions resulting from 5 and 10 milli-units. Ordinates and abscissæ as in fig. 1. For further description see text.

between the effects of another emotional stimulus of the same strength and duration and the effects of an injection of 20·0 milli-units: here it is seen that the inhibition from emotional stress is less than that from 20 milli-units. More detailed assay was not always possible when the stress caused as large an inhibition as in this dog, because the difference between the inhibitions from doses of 5, 10, and 20 milli-units—a slightly increasing delay in recovery of the rate of urine-flow with increasing dose—may be very small. In the example given in fig. 5 a more accurate assay could, however, be made. Fig. 5, *c*, shows, in the same series, the responses to 5 and 10 milli-units. Careful comparison reveals that the course of recovery from the inhibition produced by emotional stress in fig. 5, *a*, is almost identical with that from the inhibition produced by 5 milli-units in fig. 5, *c*: thus, 20 minutes after the stimulus the rate of urine-flow was 0·9 c.c. per min., and 20 minutes after the injection of 5 milli-units the rate was 0·85 c.c. per min. At 30 minutes after the stimulus or injection the corresponding figures were 1·0 and 1·4, and at 40 minutes 1·95 and 2·1 c.c. per min. Similarly the response to emotional stress in fig. 4, *b*, closely follows that to 5 milli-units. Another inhibition by emotional stress in the same period of 8 days lay between the responses to 5 and 10 milli-units. We conclude that in this dog the response to 40 seconds' faradic stimulation was the liberation from the neuro-hypophysis of antidiuretic substance equivalent to 5–10 milli-units of post-pituitary activity.

Similar assay was made of the residual inhibition produced by the same emotional stimulus applied at intervals after the removal of the posterior lobe of the pituitary. A typical result is illustrated in fig. 6, the observations being made on the same animal as that from which the results given in fig. 5 had been earlier obtained. Fig. 6, *a*, is a comparison of a response to 40 seconds' faradic stimulation with a response to the injection of 0·5 milli-unit: the latter response is seen to be the greater. On the day before these observations were made 0·03 milli-unit had had no appreciable inhibitory effect (fig. 6, *b*), while on the day after 0·1 milli-unit produced an inhibition (fig. 6, *b*) very similar to that of the emotion. In this test, therefore, the amount of antidiuretic substance liberated by the emotional stress corresponded with less than 0·5 milli-unit, and was probably about 0·1 milli-unit. During the first 2 months after removal of the posterior lobe from this dog, ten tests of the inhibition of water-diuresis by the same emotional stimulus were made and the inhibitions assayed in this way in terms of post-pituitary extract. None of the inhibitions exceeded, and only two equalled the inhibitions produced by 0·5 milli-unit; two of the tests gave no recognizable inhibition, and the average post-pituitary equivalent of the ten tests was 0·2 milli-unit. Since the same animal in response to the same emotional stimulus liberated between 5 and 10 milli-units before operation, we infer that the functional residuum

of neuro-hypophysis after removal of the posterior lobe was about 2 to 4 per cent. of the total.

The emotional inhibitions before and after the removal of the posterior lobe have been compared in this way in four dogs, and in all four certainly more than 90 per cent. of the antidiuretic function of the neuro-hypophysis was lost as a result of this removal. In the three

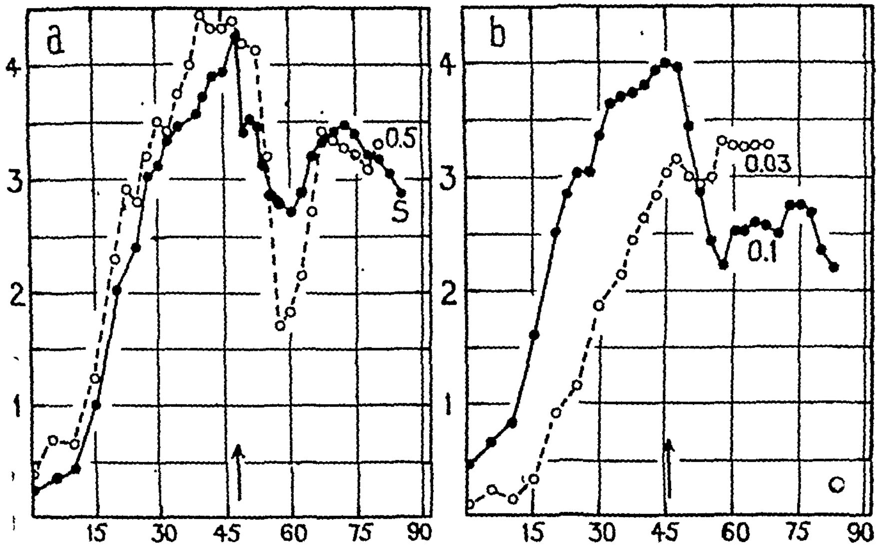

FIG. 6.—The estimation of the amount of antidiuretic substance liberated in response to 40 seconds' emotional stimulus of the same strength and with the same bitch as in fig. 5, but 1 month after removal of the posterior lobe. (*a*) The inhibition resulting from the emotional stimulus is compared with that from 0·5 milli-unit injected during the curve of the previous day. (*b*) The inhibitions from 0·1 milli-unit on the day after the curves of (*a*) and from 0·03 milli-unit on the day before the curves of (*a*). Ordinates and abscissæ as in fig. 1.

animals in which a more accurate assay was possible, only between 2 and 7 per cent. of the original function remained: the posterior lobe contained, therefore, about 95 per cent. of the antidiuretically active neuro-hypophyseal tissue.

Two of the dogs were kept without further operative interference for five months, and during this period no increase in the size of the residual inhibition of diuresis by emotional stress was observed: an indication that no regeneration or hypertrophy was occurring in the residuum of neuro-hypophysis.

DISCUSSION.

The results reported in this paper show that the posterior lobe of the pituitary liberates, in response to an adequate physiological stimulus, effective amounts of a substance which inhibits the output

between the effects of another emotional stimulus of the same strength and duration and the effects of an injection of 20·0 milli-units: here it is seen that the inhibition from emotional stress is less than that from 20 milli-units. More detailed assay was not always possible when the stress caused as large an inhibition as in this dog, because the difference between the inhibitions from doses of 5, 10, and 20 milli-units—a slightly increasing delay in recovery of the rate of urine-flow with increasing dose—may be very small. In the example given in fig. 5 a more accurate assay could, however, be made. Fig. 5, *c*, shows, in the same series, the responses to 5 and 10 milli-units. Careful comparison reveals that the course of recovery from the inhibition produced by emotional stress in fig. 5, *a*, is almost identical with that from the inhibition produced by 5 milli-units in fig. 5, *c*: thus, 20 minutes after the stimulus the rate of urine-flow was 0·9 c.c. per min., and 20 minutes after the injection of 5 milli-units the rate was 0·85 c.c. per min. At 30 minutes after the stimulus or injection the corresponding figures were 1·0 and 1·4, and at 40 minutes 1·95 and 2·1 c.c. per min. Similarly the response to emotional stress in fig. 4, *b*, closely follows that to 5 milli-units. Another inhibition by emotional stress in the same period of 8 days lay between the responses to 5 and 10 milli-units. We conclude that in this dog the response to 40 seconds' faradic stimulation was the liberation from the neuro-hypophysis of antidiuretic substance equivalent to 5–10 milli-units of post-pituitary activity.

Similar assay was made of the residual inhibition produced by the same emotional stimulus applied at intervals after the removal of the posterior lobe of the pituitary. A typical result is illustrated in fig. 6, the observations being made on the same animal as that from which the results given in fig. 5 had been earlier obtained. Fig. 6, *a*, is a comparison of a response to 40 seconds' faradic stimulation with a response to the injection of 0·5 milli-unit: the latter response is seen to be the greater. On the day before these observations were made 0·03 milli-unit had had no appreciable inhibitory effect (fig. 6, *b*), while on the day after 0·1 milli-unit produced an inhibition (fig. 6, *b*) very similar to that of the emotion. In this test, therefore, the amount of antidiuretic substance liberated by the emotional stress corresponded with less than 0·5 milli-unit, and was probably about 0·1 milli-unit. During the first 2 months after removal of the posterior lobe from this dog, ten tests of the inhibition of water-diuresis by the same emotional stimulus were made and the inhibitions assayed in this way in terms of post-pituitary extract. None of the inhibitions exceeded, and only two equalled the inhibitions produced by 0·5 milli-unit; two of the tests gave no recognizable inhibition, and the average post-pituitary equivalent of the ten tests was 0·2 milli-unit. Since the same animal in response to the same emotional stimulus liberated between 5 and 10 milli-units before operation, we infer that the functional residuum

of the antidiuretic activity of the neuro-hypophysis, as assessed in the emotional inhibition of water-diuresis, can be abolished without producing such an impairment in the animal's ability to conserve its tissue-water as would be expressed in a permanent increase in urine-flow.

We have been unable to detect any change in the sensitivity of the kidney to post-pituitary extract as a result of removal of the posterior lobe. The smallest doses which produced a recognizable inhibition during water-diuresis in four animals before and after this removal are given in Table I.

TABLE I.

Weight of animal in kg.	Minimal effective dose in milli-units.	
	Before hypophysectomy.	After hypophysectomy.
8·4	0·03–0·1	0·03–0·1
8·7	<0·05	0·02
9·7	0·05	0·05
11·2	0·05	0·05

It seems that after posterior hypophysectomy the remaining fragment of neuro-hypophysis is functionally adequate, and that only if this were removed or caused to degenerate by section of the hypothalamico-hypophyseal tracts or by destruction of their nuclei would persisting diabetes insipidus appear. On this view our observation of a residual inhibition would harmonize with the work of Fisher, Ingram, and Ranson [1938], who have shown that in the cat and in the monkey the occurrence of diabetes insipidus is contingent upon the complete degeneration or removal of the neuro-hypophysis; and it gives additional interest to the claim by Heinbecker and White [1941] that in the dog there is a correlation between the degree of degeneration in the supra-optic nucleus and the degree of increase in the daily output of urine after operative interference in the hypothalamico-hypophyseal region. Heinbecker and White [1941] state that simple removal of the posterior lobe and pars distalis is followed by a loss of from 60 to 80 per cent. of the nuclear cells, and that only when the residuum of normal cells falls to some 10 per cent. of the normal does an increase in the output of urine appear.

SUMMARY.

1. The inhibition of water-diuresis by emotional stress in the dog is largely reduced after removal of the posterior lobe of the pituitary body.

of water by the kidney during the diuresis following the administration of water by mouth. Assay of the quantity of hormone liberated in response to short-lived emotional stress shows this to be of the order of a few milli-units, and in all our experiments 1 milli-unit has been sufficient to reduce the urine-flow during water-diuresis to the resting level. Under physiological conditions, therefore, there is no need to postulate that amounts greater than this order are ever circulating in the blood-stream; and so far as we are aware there is no test which is sufficiently sensitive and accurate to detect variations in the concentration of the substance in the blood under such conditions. The rat-test described by Burn [1931] does not detect quantities much less than 20 milli-units; and, working with the rabbit, Walker [1939] has shown that the minimum dose which can be detected by this method is between 0·2 and 0·5 milli-unit. Obviously such tests are not sufficiently sensitive to detect the difference in the post-pituitary antidiuretic activity of blood-samples of, say, 20 c.c. taken from the dog at resting and diuretic rates of urine-flow seeing that, even on the assumption that the hormone is restricted to the blood-stream and not electively adsorbed at its site of action in the tubule epithelium, this difference need be only of the order of 0·03 milli-unit. Gilman and Goodman [1937] claim that when rats are deprived of food and water for 24 to 48 hours an antidiuretic substance of pituitary origin appears in the urine, but Walker [1939] has been unable to confirm this. Be that as it may, the failure to detect such antidiuretic activity in body fluids is not evidence against the control of water excretion by the neuro-hypophysis, in view of the smallness in amount of substance needed in the blood to cause antidiuresis. For the present, as seems to us, the strongest evidence of the true hormonal character of the pituitary antidiuretic substance is the demonstration of its liberation during emotional stress in the living dog and of its liberation [Pickford, 1939] by the intravenous injection of acetylcholine. We may, then, regard this substance as the normal transmitter of activity between the nerve elements in the neuro-hypophysis and the effector cells in the kidney tubules, and as a reminder of the phylogenetic history of the vertebrate excretory organs (see Gaskell [1908]).

In our experiments removal of the posterior lobe was followed by an increase in the daily output of urine from a preoperative value of about a quarter of a litre to a value of one to two litres for the first two to four days. The increase then gradually subsided, and the output of urine was apparently normal from about a week after the operation. This temporary polyuria has been observed by Fisher, Ingram, and Ranson [1938] working on the cat, and by Pickford [1939] working on the dog. We have not as yet determined whether the degree of tissue hydration subsequent to the temporary polyuria is permanently lowered, but it is remarkable that as much as 95 per cent.

of the antidiuretic activity of the neuro-hypophysis, as assessed in the emotional inhibition of water-diuresis, can be abolished without producing such an impairment in the animal's ability to conserve its tissue-water as would be expressed in a permanent increase in urine-flow.

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SUMMARY.

1. The inhibition of water-diuresis by emotional stress in the dog is largely reduced after removal of the posterior lobe of the pituitary body.

2. Consideration is given to the method of assaying the post-pituitary antidiuretic hormone by intravenous injection during water-diuresis.

3. Comparison of the post-pituitary extract equivalent of the emotional inhibition before, with that after, removal of the posterior lobe shows that only about 5 per cent. of the antidiuretic function of the neuro-hypophysis, as expressed in the inhibition by emotional stress, remains when the posterior lobe has been removed.

4. This residuum of function is apparently adequate to constrain the output of urine within normal limits.

5. The bearing of these results on the neuro-hypophyseal control of water-excretion is discussed.

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